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A molecular and morphological investigation of the red seaweed genus Porphyra (Bangiales, Rhodophyta) in the northwest Atlantic

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A MOLECULAR AND MORPHOLOGICAL INVESTIGATION OF THE RED SEAWEED GENUS *PORPHYRA* (BANGIALES, RHODOPHYTA) IN THE NORTHWEST ATLANTIC

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

A MOLECULAR AND MORPHOLOGICAL INVESTIGATION OF THE RED SEAWEED GENUS *PORPHYRA* (BANGIALES, RHODOPHYTA) IN THE NORTHWEST ATLANTIC

by

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To evaluate the possible occurrence of cryptic *Porphyra* taxa in the Northwest Atlantic, extensive field collections were made during winter and spring periods when relatively few previous collections had been made. Approximately 100 different sites extending from Chance Harbor, New Brunswick, Canada to Rye, New York, USA, were sampled during multiple years (2001-2005). Historical specimens from several herbaria (NHA, FH, BM, WTU, MICH, US) were also examined for possible cryptic taxa. A combination of morphological and molecular tools was used to screen both recent and historical collections.

Sequences from the plastid-encoded, ribulose-1, 5-bisphosphate carboxylase-oxygenase large subunit (*rbc*L) gene and *rbc*L-*rbc*S spacer, the nuclear ribosomal small subunit (SSU), and internal transcribed spacer 1 (ITS1) regions were compared with other *Porphyra* taxa sequences available on

GenBank. Sequences from four taxa were found to match Pacific taxa, with three of these having not been previously reported for the Northwest Atlantic (i.e. *P. rediviva* Stiller & Waaland, *P. yezoensis* f. *narawaensis* Miura, *P. katadae* Miura,). In addition, five previously undescribed taxa, with unique *rbc*L, SSU, and ITS1 sequences were discovered.

Porphyra rediviva, which was previously described from the Pacific, could not be morphologically or ecologically distinguished from *P. purpurea* (Roth) C. Agardh. Additionally, the *rbc*L and ITS1 sequences from the nomenclatural type specimens of both species were identical, providing compelling evidence for the synonymization of *P. rediviva* under the older named taxon, *P. purpurea.* As there was an equal amount of molecular variation within both the Atlantic and Pacific populations of *P. purpurea,* the designation of a parent population was unclear. With this synonym, the number of known monostromatic species occurring in the Northwest Atlantic having male/female gametangia on separate longitudinal "halves" of the blade includes *P. purpurea, P. birdiae,* and *P. katadae.* While molecular differences among these three species are distinct, morphological and ecological features are less clear. However, differences between these taxa are more apparent if combinations of both morphological and ecological characteristics are used. For example, by combining blade thickness and color, *P. katadae* can be delineated from both *P. purpurea* and *P. birdiae,* while a comparison of distributional patterns show a clear distinction between more northern *P. birdiae* and southern *P. katadae.*

Despite the recent report of *Porphyra yezoensis* f. *yezoensis* from a single site in New Hampshire, USA, molecular screenings of specimens from across the region provides strong evidence of a once continuous distribution from mid-Maine to western Long Island Sound. In addition to the populations of *P. yezoensis* f. *yezoensis* in New England, three populations of this taxon were found on the Gulf Coast of Texas, with one specimen dating back to the late 1960's. When comparisons of ITS1 sequences were made with Asian individuals, all New England specimens plus one Texas specimen had identical sequences to Japanese specimens, while the other two Texas populations showed a closer affinity to Chinese populations. Thus, the molecular evidence supports at least two introductions of *P. yezoensis* f. *yezoensis* to the Gulf Coast of Texas. The discovery of a second Asiatic cultivar, *P. yezoensis* f. *narawaensis*, in New England appears to be of more recent introduction than the established f. *yezoensis* as documented by its later development (1980's) and more circumscribed distribution that interrupts the continuous distribution of *P. yezoensis* f. *yezoensis.*

The ITS1 sequences of New England *Porphyra yezoensis* f. *narawaensis* populations show no variation and they are identical to as many as 16 commercial strains in Japan. Similarly, the distribution of another Asiatic species discovered in New England, *P. katadae*, is circumscribed, and it appears to be a recent introduction that shows no sequence variation in either the *rbc*L gene or ITS1 region. Comparison of ITS1 sequences with Asiatic specimens show New

England specimens of *P. katadae* are identical to a specimen from China and differ by a 1 bp substitution from a specimen from Japan.

Four of the five newly discovered taxa are herein described as new species (*P. collinsii sp. nov.*, *P. stamfordensis sp. nov.*, *P. tsengii sp. nov.*, and *P. spatuala sp. nov.*). Detailed morphological and ecological descriptions are given along with their distributional pattern in New England. Sequences for partial *rbc*L, *rbc*L-*rbc*S spacer, SSU, and ITS1 are submitted to GenBank for type specimens. The fifth new species also occurs in the Mediterranean and has been recently described *(as Porphyra olivii sp. nov.)* in a joint publication with several European collaborators.

INTRODUCTION

This dissertation explores the occurrence, distribution, and possible origins of cryptic species of the red algal genus *Porphyra* C. Agardh (Bangiophycidae, Rhodophyta) in the Northwest Atlantic. Particular emphasis has been placed upon the identification, delineation, and description of unreported species of *Porphyra* based on data from morphological, anatomical, and molecular techniques. While each chapter is intended to stand alone, collectively they aim to enhance our understanding of the diversity of *Porphyra* in the Northwest Atlantic.

Chapter I presents a brief description of the Genus *Porphyra* (i.e. life history, systematics, economic importance). A synopsis of seaweed biogeography is presented with emphasis upon long-range dispersal. Particular attention is given to North Atlantic speciation as well as human mediated introductions of marine species.

Chapter II compares four morphologically similar, reproductively sectored species of *Porphyra* using traditional and molecular techniques. A comparison of morphology and DNA sequences from multiple sites (*rbc*L, ITS1, SSU) revealed the conspecificity of two of these species, *Porphyra purpurea* (Roth) C. Agardh and *P. rediviva* Stiller & Walaand, with the former having taxonomic priority. As a result of this synonymization, the distribution of *P. purpurea* is now confirmed from both the North Atlantic and North Pacific Oceans. A synopsis of

morphological, anatomical, ecological, and temporal characters is summarized to delineate morphologically similar species occurring within the Northwest Atlantic. A manuscript based on this chapter in its entirety has been submitted to *Nova Hedwigia* as: Bray, T. L., Neefus, C. D. and Mathieson A. C., A morphological and molecular investigation of the *Porphyra purpurea* complex in the Northwest Atlantic.

Chapter III describes the occurrence of three introduced Asiatic *Porphyra* taxa on the New England coast (*P. yezoensis* f. *yezoensis* Ueda, *P. yezoensis* f. *narawaensis* Miura and *P. katadae* A. Miura), as well as three sites along the Gulf Coast of Texas (*P. yezoensis* f. *yezoensis*). *Porphyra yezoensis* forma *narawaensis* Miura and *P. katadae* A. Miura are reported from North America for the first time. Sequence data reveals a close affinity between Asian and North American specimens, suggesting recent human-mediated introductions. Sequences from the non-conserved region (ITS1) revealed the occurrence of two different strains of *P.yezoensis* in New England suggesting multiple introductions. A comparison of ITS1 sequences from New England and Asian samples also suggests multiple sites of origin. Both strains of *P. yezoensis* found in New England show close affinities to Japanese specimens. One strain matches the GenBank sequence of a wild specimen of *P. yezoensis* f. *yezoensis* from Hokkaido, Japan, while the other is identical to commercial cultivars of *P. yezoensis* f. *narawaensis* that are extensively grown via aquaculture along the coast of Japan, Korea, and China. Several specimens of *P. yezoensis* f. *yezoensis* were also collected from the Gulf Coast of Texas. While one Texas

populations had identical ITS1 sequences to New England populations, the other two showed a closer affinity to Chinese specimens. Detailed geographical information is provided to document the spread and impact of invasive *Porphyras* in New England. A portion of this chapter appears in a manuscript submitted to *The Journal of Phycology*: Neefus, C. D., Mathieson, A.C., and Bray, T. L., The occurrence of three introduced Asian species of *Porphyra* (Bangiales, Rhodophyta) in the northwestern Atlantic.

Chapter IV describes four new species of *Porphyra* for which no matching sequences or descriptions are known: *P. collinsii sp. nov., P.tsengii sp. nov., P. spatulata sp. nov.,* and *P. stamfordensis sp. nov.* Morphological, anatomical, ecological, seasonal, and molecular characters of the species are provided. Although unique plastid (*rbc*L) and nuclear (ITS1, SSU) sequences are reported for each species, phylogeographical analysis of the newly described species reveals a closer affinity to Asiatic and Mediterranean species than to endemic North Atlantic species suggesting possible recent introductions. Based upon findings reported in Chapter III and the fact that less than half (~ 60) of the described species of *Porphyra* have sequences available in GenBank, these taxa could represent previously described Asian species never before reported in New England. A fifth newly described species of *Porphyra, P. olivii,* is reported from New England along with its description and distribution pattern. This portion of Chapter IV is included in the article submitted to *The European Journal of Phycology*, J. Brodie, I. Bartsch, C. Neefus, S. Orfanidis, T. Bray, and A. Mathieson New insight into the cryptic diversity of the North Atlantic-

Mediterranean '*Porphyra leucosticta*' complex: *P. olivii* sp. nov. and *P. rosengurttii* (Bangiales, Rhodophyta).

CHAPTER I.

SEAWEED BIOGEOGRAPHY

Introduction

Porphyra is a blade-forming red algae that grows on rocky, cold-to-warm-temperate shorelines throughout the world (Brodie et al. 1996, Yoshida et al. 1997). Due to its economic value, *Porphyra*, or "nori," is one of the most highly investigated seaweeds. In Japan alone, its harvests yield about 10 billion sheets for human consumption, with an estimated annual production value of 1.8 billion U.S. dollars (Jensen 1993). Additionally, increased production of nori in the Republic of Korea and China has made its production the largest component of a 5.5-6.0 billion dollar global seaweed industry (McHugh 2003, Saga & Kitade 2002). Within the human diet, *Porphyra* has been shown to be a good source of taurine (Noda et al. 1975) that controls blood cholesterol levels (Tsujii et al. 1983). Other uses of *Porphyra* include a commercial source of the red pigment *r*-phycoerythrin, which is used as a fluorescent "tag" for DNA and microscopic evaluations (Mumford & Miura 1988).

Generally, *Porphyra* has a biphasic life history alternating between a foliose gametophyte and a microscopic, shell-boring sporophtye or "conchocelis" phase (Brodie and Irvine 2003, Mitman & van der Meer 1994). Different species

of *Porphyra* can be monoecious, dioecious, or both (Brodie & Irvine 2003). Thalli can be 1-2 cells thick and they grow attached to rocks, shellfish, or other seaweeds within the intertidal or shallow subtidal of subartic to the subtropic regions.

Porphyra belongs to the family Bangiaceae, which also includes the genus, *Bangia.* Currently, 140 species of *Porphyra* are recorded (Silva 1999, Yoshida et al. 1997). However, recent taxonomic investigations (Brodie & Irvine 1997, Farr et al. 2003, Lindstrom & Fredericq 2003, Neefus et al. 2002, Nelson et al. 2006) suggest that the number of species may be much greater. Historically, the taxonomy of *Porphyra* has been largely based on blade morphology, including color, size, shape, thallus thickness, and the distribution of fertile thallus tissue. Due to its long evolutionary history some morphological characters have been shown to be homoplastic, such as blade shape and the distribution of reproductive areas of the thallus (Lindstrom & Fredericq 2003, Nelson et al. 2006), which has lead to some taxonomic confusion and misidentifications.

Clarification of phylogenetic relationships for many *Porphyra* species has occurred using the nuclear ribosomal small subunit (SSU) and the chloroplast ribulose bisphosphate carboxylase (*rbc*L) genes (Broom et al. 2002, Far et al. 2003, Klein et al. 2003, Kunimoto et al. 1999, Lindstrom & Fredericq 2003, Nelson et al. 2006, Oliveira et al. 1995). In attempting to refine species boundaries, noncoding regions such as the nuclear internal transcribed spacer (ITS) and the ribosomal introns have been used (Broom et al. 2002, Kunimoto et al. 1999, Teasdale 2004).

Understanding distributional patterns of various *Porphyra* taxa can be a difficult task due to a variety of factors. First, the complexity of marine environments has retarded historical understanding and delineations of marine provinces. Second, the long history of *Porphyra,* 2 billion-500 Ma (Campbell, 1980, Tappan 1976, Xiao et al., 1998), spans numerous global geological events, obscuring possible points of origin. Third, the persistence of *Porphyra* with little apparent morphological changes over this period suggests its capacity to survive a variety of intertidal environments and large-scale climate changes, making it difficult to accurately delineate its major marine boundaries (Broom et al. 2004). Fourth, the morphological simplicity and paucity of characteristics used to distinguish *Porphyra* species has contributed to a global underestimation of species (Bird & McLachlan 1992, Broom et al. 2004, Jones et al. 2004, Lindstrom & Fredericq 2003, Neefus et al. 2002, Nelson et al. 2006); thus, reported distributions of a single species may actually comprise multiple cryptic taxa. Fifth, contemporary global economies continue to provide vehicles for daily introductions and re-introductions of marine species around the world (Carlton & Geller 1993, Fofonoff et al. 2003, Ruiz et al. 2003); such convoluted pathways make the assessment of distributional ranges challenging and contribute to the dynamics of local floras.

In recent years, molecular tools have become powerful instruments in understanding and reconstructing the biogeography of *Porphyra.* The development of genetic markers has also helped to identify patterns of genetic structure and gene flow within/among extant populations at both the regional and

local scale (Broom et al. 2004, Kunimoto et al. 1999a, 1999b, Niwa et al. 2005, Teasdale 2004). Sequence data has been used to reveal unexpected species richness in various geographical regions previously reported to have low species diversity (Broom et al. 2004, Jones et al. 2004).

Historical Synopsis of Seaweed Biogeography

An historical context is important in attempting to understand biogeographical patterns of various *Porphyra* taxa. The delineation of marine biogeographic provinces has lagged behind those for terrestrial habitats. Setchell (1917) noted the difficulties of collecting within diverse geographies, as well as the lack of economic value placed upon seaweeds. Van den Hoek and Donze (1967) argued that in several earlier attempts to define algal phytogeographic provinces, too much was taken for granted. Druehl (1981) listed four problems associated with adequate phytogeographic evalutions: 1) sparseness of complete floristic records; 2) proof of discontinuous distributions; 3) uniformity of taxonomic criteria; and 4) the major assumption that seaweed distributions are static.

The first efforts at describing geographical distributions of marine plants were floristic and largely taxonomic (Goodenough & Woodward 1797, Turner 1802, C. A. Agardh 1817, Lyngbye 1819, Le Jolis 1864, Farlow 1881, Collins 1900, Skottsberg 1906). Lamouroux (1825) outlined several factors that affected the distribution of marine algae, including water depth, temperature, light, aeration, and substratum. His work laid the foundation for several

biogeographical studies, including those of Greville (1830), Harvey (1849), Kjellman (1883), Reinke (1889), Börgesen (1902), Simmons (1906), Jönsson (1912), and Setchell (1915).

Comparisons of local marine floras have been investigated on a global scale (Yendo 1902, Saunders 1901, Setchell & Gardner 1903, Schroeder 1912). In the North Atlantic, Cape Cod, Massachusetts, and Cadiz, Spain, are major phytogeographic boundaries delineating northern and southern species (Harvey 1858, Setchell 1915). In Japan, Yendo (1902) considered Cape Inuboi on the east coast of Honshu and the Strait of Sangar as sites of major marine floristic demarcation, with the distribution of Japanese *Porphyras* reflecting this floristic boundary (Miura & Aruga 1987). Point Conception, California and the Gulf of California were also major points of floristic demarcation (Saunders 1901; Setchell & Gardner 1903). Pielou (1977) states that zonation can also influence seaweed distributions. The complexity of environments, including temperature and salinity variability (temporally and spatially) can also add to difficulties delineating algal phytogeographic provinces. For example, Collins (1908) described isolated "warm water" sites along the New England coast where "southerly" plants occurred.

Long-range Dispersal of Seaweeds

The occurrence of seaweeds on the margins of recently established volcanic islands provides compelling evidence that long-range dispersal of seaweeds can occur in a relatively short period of time and apparently against

the prevalent direction of currents (Jönsson 1970, van den Hoek 1987). Spores of benthic algae have been reported in pelagic habitats (Amsler & Searles 1980, Hruby & Norton 1979, Zechman & Mathieson 1985). However, the establishment of dioecious species of seaweeds (*e.g. Macrocystis*) by long-range dispersals of unicellular propagules seems unlikely, as it would require chance dispersals of two separate gametophyte generations. Moss et al. (1981) speculated that long-distant dispersals of microscopic female gametophytes containing a few-celled sporophyte were responsible for the occurrence of *Laminaria* populations on North Sea oil platforms. Based upon microsatellite assessments, Coyer et al. (2006) argued that the common intertidal seaweed, *Fucus serratus*, was introduced to Iceland and the Faroes by human settlers from Europe. Zechman and Mathieson (1985) found that most red algal spores seem to rapidly sink providing a greater opportunity for fixing quickly to the substratum and limiting dispersal range. An exception among red algae would be the genus *Porphyra,* which produces buoyant spores (Suto 1950).

Dispersal of multicellular seaweed fragments and propagules has been observed at great distances from the nearest shore (Norton & Mathieson 1983, Woelkerling 1975). For example, the northern fucoid algae *Ascophyllum nodosum* (L.) Le Jolis and *Fucus vesiculosus* L. are regularly recorded from the Sargasso Sea, with these often bearing various algal epiphytes (Woelkerling 1975), including some species of *Porphyra* (Bird & McLachlan 1992, Brodie & Irvine 2003, Taylor 1957). John (1974) reported floating fragments of *Ascophyllum* propagules near the equator off the West African coast. However,

the vast majority of algae cannot float and their transport would typically be limited to epiphytic populations or floating objects (van den Hoek 1987).

Jokiel (1984) reported trans-Pacific dispersal of seaweeds via vectors such as net floats from Japan and floating pumice from San Benedicto Island, Mexico. Another possible vector in long-range dispersal and one that also lacks adequate study, is via marine animals. Spores and fragments of adult plants could be carried by foraging sea birds to remote locations. Some seaweeds, such as the conchocelis phase of *Porphyra,* spend part of their life cycle within barnacles that can grow attached to whales and marine turtles that tend to migrate long distances.

Seaweed Speciation in the North Atlantic

Major geological events during the last 500 million years would have greatly affected global distribution and speciation of various *Porphyra* taxa. The net migration of biota from the North Pacific to the North Atlantic is considered commonplace by zoologists (Briggs 1974, Franz & Merrill 1980). Disparity in the number of endemic algal taxa between the North Pacific and North Atlantic, as well as the apparent ability of North Pacific taxa to invade the Arctic during the late Pliocene, has led some phycologists to speculate that North Pacific species might have provided the "seed" stock for closely related or sibling taxa in the Arctic and North Atlantic (Lindstrom 1987, Lindstrom 2001, Lindstrom & Cole 1992, Lüning 1990). Based on morphology, cytology, isozymes, and molecular evidence, several closely related North Atlantic and North Pacific species-pairs of

Porphyra such as *P. amplissima*/*P. cuneiformis* and *P. miniata*/*P. variegata* have been reported (Lindstrom & Cole 1992, Lindstrom & Fredericq 2003, Stiller & Waaland 1996).

The breakup of Pangaea dates back some 200 Ma (Fallow 1979, Fallow & Dromgoole 1980). The opening of the South Atlantic Ocean began with the separation of West Gondwana to form the African and South American continents and the formation of tropical marine regions such as the Central Atlantic, Caribbean, and Gulf of Mexico. The North Atlantic was not formed until 165 Ma as North America began to separate from the European plate (Kennett 1982).

It has been suggested that the western Arctic Ocean (sometimes called the Boreal Gulf) served as a northern embayment to the Pacific Ocean during Mesozoic times (Lawver et al. 1990). Surface water temperatures in the late Mesozoic Arctic were upwards of 14°C warmer than present day (Emiliani 1961), which would suggest that the ancestors of present day species had more northern distributions. Mesozoic oceans would have provided three distinct temperature zones for algae: (1) the warm tropical Tethys Sea; (2) the tropical to temperate Pacific; and (3) the coolest Arctic waters (Valentine 1973).

By the end of the Cretaceous (ca. 100 Ma), the Pacific-Arctic biotic continuity ended with the closure of the deep waterway connecting the Arctic and North Pacific oceans and concurrent opening of the North Atlantic (Pitman & Talwani 1972). North Pacific species became isolated from the Arctic Ocean by the Bering land bridge and were restricted in the south by warm tropical waters

(van den Hoek 1984). Lüning (1990) suggested that it was during this 60 million year period of isolation that the North Pacific became the primary area for the evolution of cool water endemic taxa. During this same period, the smaller Arctic Ocean provided an important environment for biotic innovations and became the ancestral birthplace of major groups that would later radiate to lower latitudes (Hickey et al.1983). Early phycologists (Kjellman 1883, Simmons 1906) expressed a similar view regarding the Arctic Ocean.

While the ancient Arctic Ocean was never completely isolated because of the existence of the Turgai Strait from western Siberia to the Tethys Sea during the early Tertiary (Hallam 1981, Marincovich et al. 1990), major Arctic Ocean connections with the North Atlantic (while still closed off from the North Pacific) began around 40 Ma during the mid-Eocene (Srivastava 1985, van Oppen et al. 1995). Coinciding with the flow of cooler Arctic waters into the North Atlantic was a global drop in seawater temperature (Lüning 1990, van Oppen et al. 1995, Van Andel 1985). Cooling ocean temperatures provided opportunities for newly introduced Arctic species to inhabit coastlines replacing retreating warm water taxa and to evolve into more temperate species. Seaweed species migrating southward from the Arctic Ocean via the Norwegian-Greenland Sea colonized both sides of the North Atlantic (van den Hoek 1984).

It was not until the Late Miocene-Late Pliocene (5-2 Ma) that the North Pacific and North Atlantic became reconnected via the Arctic Ocean allowing the exchange of cool temperate genera to resume after 60 million years (Hallam 1994, Lüning 1990, van den Hoek 1984). The opening of the Bering Strait

coincided with a significant temperature drop during a major series of warming and cooling phases that preceded the glacial-interglacial events of the Pleistocene. Lüning (1990) suggested that these series of temperature changes sent migration pulses back and forth across the Arctic Ocean. Such migrations would probably have excluded those species with their northernmost limits in the Boreal region and included those with an Arctic to temperate distribution (van den Hoek 1975). Hallam (1994) reported that over 125 animal species (mainly bivalves and gastropods) entered the Arctic-Atlantic region from the Pacific during the Late Miocene, while only 16 species of North Pacific species are of Atlantic origins. Such asymmetry in species migration is not unique. Por (1978) reported that nearly all invaders passing through the Suez Canal came from the Red Sea into the Mediterranean— *i.e.* they exhibited a Lessepsian distribution.

Vermeij (1991) summarized five hypotheses to explain this asymmetrical trans-Arctic invasion. First, the Null Hypothesis of Diversity predicts that the number of invading species is proportional to the number of species in the source biota before the exchange. Thus, according to this hypothesis the greatest biological diversity would have been in the North Pacific. Second, the Null Hypothesis of Area states that the dominate donor biota is the one with the largest number of individuals. Third, the Hypothesis of Circulation predicted that the movement of Arctic-Atlantic species to the Pacific was unfavorable due to current circulation patterns. Fourth, the Hypothesis of Ecological Opportunity explained the asymmetric establishment of Pacific species into the Arctic-Atlantic in terms of environmental conditions in the region of the recipient biota. If

conditions prior to the exchange were more extreme in the North Atlantic and extinctions occurred, invading species would have had a greater opportunity to gain a foothold. Fifth, the Hypothesis of Competitive Dominance argues that successful invaders exhibit a reproductive, competitive, and defensive superiority. Vermeij (1991) is careful to point out that these five hypotheses are not mutually exclusive and other explanations could exist.

During the Pleistocene (2 Ma-18,000 years ago) there were upwards of 20 glacial and interglacial periods that caused sea levels to oscillate, resulting in repeated submergence and re-emergence of the Bering Land Bridge (Hopkins 1967). Marine migration patterns would have been greatly disrupted by this fluctuating land barrier. While the Pacific became separated from the Arctic Ocean by the emergence of the Bering Land Bridge, the North Atlantic and Arctic Oceans remained in full contact. This open exposure to the Arctic Ocean and the relatively small size of the North Atlantic caused pronounced marine temperature fluctuations (van den Hoek 1975, McIntyre 1976). Such instability of marine climate in the North Atlantic during the Pleistocene has been offered as one possible reason for higher diversity of marine algae occurring in the North Pacific compared to the North Atlantic (van den Hoek 1975, 1984). In addition to the emergence of the Bering Land Bridge, perennial sea-ice cover of the Arctic Ocean would have blocked any further migration of North Pacific taxa into the North Atlantic via its waterways. Estimates for perennial ice cover range from 0.7 Ma (Herman and Hopkins 1980) to 3 Ma. (Clark 1982).

During the height of the last great ice age (18,000 years ago) the eastern and western boundaries of the North Atlantic were subjected to different temperature stresses (Breeman and Pakker 1994). On the North American side of the Atlantic Ocean water temperatures fluctuated less between winter and summer, thus Breeman (1989) suggested that southern boundaries of northern seaweeds would have exhibited little change since typically northern species are more limited by the requirement for lower temperatures for reproduction than by higher summer temperatures. Pack ice during the last glacial maximum reached as far south as the British Isles (ca N51° latitude) on the eastern side, resulting in a latitudinal shift of benthic algae of about 15-20° latitude (van den Hoek 1975). In contrast, on the western side of the North Atlantic Long Island Sound (ca N40° latitude) was the southern extent of glacial pack ice resulting in only a shift of about 5-10° (Ingolfsson 1992, Lüning 1990, van den Hoek 1975, 1984, van Oppen et al. 1995).

With the advancing ice pack many western Atlantic species requiring a rocky substrate would have found little suitable habitat beyond the southern most extent of the glaciers at Long Island Sound (Knott & Hoskins 1968, Riggs et al. 1996, Searles 1984, Teasdale 2004, van den Hoek 1975). Due to this absence of suitable habitat to the south, it has been proposed that rocky intertidal communities of New England experienced a high degree of extinction; thus some extant species are the result of migration and colonization from Europe during the last 13,000 years where greater expanses of hard substrata were available and climatic changes were not as severe (Ingolfsson 1992, Wares and

Cunningham 2001). In comparing European and North American haplotypes of *Porphyra umbilicalis,* Teasdale (2004) presented evidence that was consistent with the hypothesis that North American populations were the result of European colonization.

However, the recolonization of northwestern Atlantic coastlines by surviving European floras is not universally accepted. The SW-to-NE direction of the Gulf Stream and North Atlantic Drift would have created a formidable obstacle to such transoceanic dispersal (Wilce unpubl., in South, 1983). The question of trans-Atlantic dispersal and the uncertainty of just how inhospitable mid-Atlantic coastlines were in North America during glacial advances when sea levels were lower (~ 130 m) has led to alternate explanations for the high resemblance between northeastern and northwestern Atlantic floras. For example, van den Hoek (1984) considered northeastern and northwestern Atlantic floras as simply vicariant portions of a once continuous flora. While suggesting local Northwest Atlantic *Phycodrys rubens* might have become extinct during periods of glaciations, van Oppen et al. (1995) argued that the source for recolonization was the Pacific or Arctic regions rather than European populations.

Human Mediation of Introductions

While most of the global biogeographic patterns observed today can be linked to past events such as fragmentation of continents, emergence of land barriers, and extreme shifts in global climate (i. e. glaciation events), increasingly

greater numbers of distributional patterns are the result of more contemporary anthropogenic events. The transportation of organisms to new locations via human-mediated vectors (i. e. ship hulls, ballast water, shellfish importation) and the construction of suitable substrata (i. e. docks, sea walls, bridges) and artificial pathways (canals) provide some species opportunity to expand their geographical ranges.

Over the last 700 years the most probable vehicle for introductions of various marine plants has been by humans. In the fourteenth century, Marco Polo's overland expedition inflamed the appetites of western Europeans for the exotic commodities of the Far East. The prospect of wealth and power caused many nations to subsidize intrepid explorers in finding faster and more efficient routes to the Orient. The introduction of European ships into the Indian and Pacific Oceans began in the early 16th century with Portuguese explorers such as Magellan and Albuquerque. Ocean trade routes were established to eastern markets and powerful trading companies were founded, such as the Dutch and English East India Companies. Ships became inadvertent conveyors of plants by serving as flotsams and living rafts (Mack 2003). By the early eighteenth century a triangular trade route was established between Europe, West Africa, and the Caribbean following the weaker Canary Current off North Africa, the westerly South Equatorial Current, and returning with the Gulf Stream (Viola and Margolis 1991).

The establishment of American colonies served to expand the market for eastern delicacies and European goods as trans-Atlantic shipment entered ports

such as Boston and New York. There were more than 235 vessels utilizing the harbors of New York and Boston by the early 1700's (Anonymous 1941). After enduring eight years of war for independence and barely three months after the last British war ship left New York Harbor, the *Empress of China* set sail on February 22, 1784 for the first American voyage to China (Albion 1970). By the 1790's American trade with China entailed regular stops to the American Northwest coast where furs could be obtained for trinkets and traded in Canton for tea, silk, and chinaware. Profitable trade, which also included sandalwood from Hawaii, was monopolized by large Boston firms (Albion 1970). In 1835 the number of vessels arriving monthly in New York, Boston, and Philadelphia from Indian and Pacific Ocean ports was 41, 29, and 10, respectively (Albion 1970). Perry's successful negotiations with Japan in 1854 opened these ports to the United States and led the way for additional treaties with Western Europe and Russia (Howe 1996). In 1860 trade goods entering New York and Boston from countries in the Indian and Pacific Oceans exceeded 100,000 tons (Albion 1970).

With the advent of steamships, a network of oceanic pathways soon became established. Trade routes that once relied upon ocean and wind currents were replaced by more direct and economically strategic routes made possible by the establishment of coaling stations. By 1889 Britain alone had 156 such stations spanning the world (Porter 1991). Dockings at these British coaling stations along with those of Germany, Portugal, and Spain, ships introduced immigrant plants to remote locations where natural introductions would have been highly unlikely. For example, Ascension and St. Helena Islands in the

southern Atlantic became the recipients of many accidentally introduced plants (Cronk 1989).

One of the earliest modes for the accidental dispersal of plants via shipping was ship ballast, which consisted of rubble, gravel, stones or any other dense debris that was dumped along the shore before outbound cargo was loaded (Mack 2003). The material was gathered indiscriminately at the ports of embarkation and often included a variety of coastal plants, which was evidenced by numerous plants seen growing atop the ballast heaps by early biologists (Martindale 1877). Wharves, piers, and ballast dumping grounds became unique collection sites for many new species. Rhoads and Klein (1993) reported 81 species in the Pennsylvania flora that had only been found on ballast heaps around the Philadelphia harbor; none of these species have been collected in that State in more than a hundred years. While it is not surprising that many adventive vascular plants did not survive in their new environments for extended periods, a few survived. Thompson (1991) reported that *Spartina alterniflora* Loisel, one parent of the aggressive hybrid *Spartina anglica* C.E. Hubb, now invasive in salt marshes in England, was transported from northeastern North America to Britain as seed in solid ballast. Mack (2003) proposed that ballast plant dispersal could also explain the early misconception about the taxonomic affinities among tropical floras. Mack (2003) contended that Linnaeus' conclusion that tropical floras worldwide were strikingly similar was due to collectors' inability or unwillingness to venture into the tropical interior, thereby,

confining their plant collecting to seaports, which often shared common floras transported by humans.

With the advent of mechanical pumps, seawater quickly replaced solid ballast in ships by 1900. Water pumped from one port was commonly jettisoned into another without regard to the possible introduction of non-indigenous marine life (Carlton and Geller 1993). In the U.S. alone commercial vessels arriving from outside the country are estimated to be 50,000 per year (Ruiz et al. 2001). An estimated 80 million metric tons of ballast water may be discharged from vessels docked in U.S. waters (Carlton et al. 1995) and up to 3,000 million tons worldwide (Gollash 1996). Once introduced, non-indigenous species can be spread along the coast via domestic shipping that is shorter in duration (12-72hr) than transoceanic voyages (6-30 days), allowing greater survival rates within ballast tanks and providing a major source for coastwise transport and spread of non-indigenous species (Hines et al. 2004). Approximations of ballast water discharges from domestic arrivals in New England appears to be nearly 35% of the total ballast water discharged by commercial ships; however, this figure could be an underestimation because there has been no federal requirement that domestic traffic report their ballast activities (Hines et al. 2004).

Since the late 1980's much attention has been given to invasions associated with ballast water because of the dramatic and catastrophic effect of zebra mussels (*Dreissena polymorpha* Pallus) and quagga mussels (*Dreissena bugensis* Andrusov) on the Great Lakes (Mills et al. 1993). Ballast systems of modern ships provide a complex array of environments that can be utilized and

colonized by organisms in ballast water (Fofonoff et al. 2003). Hallegraeff and Bolch (1992) surveyed 343 cargo vessels and found that 65% contained ballast tank sediments, which provides an ideal medium for the colonization of bottom communities that can persist for months or even years, creating opportunities to "reseed" each new batch of ballast water before discharge (National Research Council 1996). Such occurrences could help to explain the dramatic rise in the numbers of recorded invasions within North America after 1900. Fofonoff et al. (2003) reported an increase of 475% for nonindigenous sessile and sedentary organisms and an increase of 733% for nonindigenous mobile taxa in North America.

Despite recent attention to ballast water, hull fouling may still be an important vector for the invasion of non-native species (Carlton et al. 1995). Historically, hull fouling had played an important role in the dispersal of marine organisms (Carlton 1987). The wooden hulls of ancient sailing vessels provided a more favorable surface for settlement and the slower speeds (5 to 6 knots) lowered the impact on the survival of fouling organisms (Allen 1953). In an attempt to understand the role of pre-1800 vessels in dispersal events, Carlton and Hodder (1995) made observational and experimental studies on the dispersal of hull-fouling organisms on a replica of a 16th century sailing vessel, the *Golden Hinde II*. They showed that there were no ecologically significant changes in the abundance of fouling species on the ship's hull while in protected harbors versus completing open sea voyages. Additionally, there was little loss

in the overall number of taxa, with survival rates as high as 95% between dockings and open sea voyages.

In terms of modern ships, it had been assumed that hull fouling was a less important vector than ballast water because of: (1) the utilization of antifouling paints; (2) shorter stays in ports than historical predecessors; and (3) vastly increased ship speeds (Carlton et al. 1995). However, it has been shown that some taxa have now evolved resistance to copper-based antifouling paints (Hall 1981). Low-salinity species that would not survive as well under longer exposures to full-strength seawater could actually benefit from the shorter travel times of faster, modern ships (Roos 1979). Furthermore, there are still many slow-moving vessels, such as drilling platforms and floating dry docks that regularly cross the world's oceans. Carlton (1996) also suggests that recent increases in water quality for many harbors and ports could lead to more abundant and diverse communities fouling the hulls of visiting ships. With > 300 million m^2 of submerged hull surface arriving each year to U.S. ports (Ruiz et al. 2003), hull-fouling is capable of extensive transfers of many different types of taxa. In a study of 189 exotic marine species, Siguan (2003) attributed the introduction of at least 39 marine plants to hull fouling, with most of these (31) being red algae.

Fofonoff et al. (2003) studied 316 nonnative species of invertebrates and algae in coastal marine habitats of continental North America. After closely evaluating life history, ecology, invasion history, salinity tolerance, and habitat utilization, they attributed the invasions of all but 65 species (20.5%) to shipping

(hull fouling and ballast water). Across 16 different phyla, shipping was considered either the sole vector or one of multiple vectors. Fofonoff et al. (2003) showed that invasions associated with shipping occurred more often on the Pacific than the Atlantic coast. One possible explanation for such disparity is exemplified by the transport of Atlantic oysters (*Crassostrea virginica* Gmelin) to the Pacific coast, whereby many other species were inadvertently transported. In contrast, few translocations of oysters to the Atlantic and Gulf coasts from the Pacific have occurred.

In what may be the best examples of artificial pathways, the opening of both the Suez and Panama Canals linked two biogeographically distinct marine provinces that had been separated for several million years. Por (1990) estimated that 200-300 species from the Red Sea (i.e. Lessepsian taxa) have colonized the Mediterranean since the completion of the Suez Canal in 1869. Siguan (2003) reported 24 Red Sea immigrants (15 red algae, 4 brown algae, 4 green algae, 1 higher plant) in the Mediterranean. Such findings are not surprising given that 14% of the total world trade, 26% of oil exports, and 41% of the total volume of goods and cargo that arrive in Arab Gulf ports pass through the lockless Suez Canal (http://www.sis.gov.eg/calendar/html/c1171196). The opening of the 50-mile long Panama Canal in 1914 has presented less of a problem in the passage of marine species due to the saline disruptions along the canal by freshwater lakes, as well as herbivore activity and the lack of reef-generated refuge areas on the Pacific coast (Hay & Gaines 1984). Nevertheless, there have been recent implications of the Panama Canal in the introduction of

the Pacific jellyfish, *Phyllorhiza punctata* von Lendenfeld, to the Gulf of Mexico and the Caribbean black-striped mussel, *Mytilopsis sallei* Reclúz, to Australian marinas (William et al. 2003; Hutchings et al. 2002).

The introduction of exotic marine species has also been associated with modern aquaculture. Siguan (2003) attributed the introduction of 64 nonnative marine plant species to aquaculture activities. Because the microscopic sporophyte of *Porphyra* bores into and lives in calcareous substrates (Boney 1965, Hollenberg 1958, Lubchenco & Cubit 1980), the shellfish industry is particularly suspect in the spread of non-native species. Clokie and Boney (1980) reported a close link between conchocelis infected shells in inshore waters and the abundance of littoral stocks of *Porphyra* blades. Siguan (2003) described sites of shellfish culture as "hot spots" in terms of the number of exotic species found at these locations and stated that this vector is the largest single pathway for naturalized (established) populations. Verlaque (2001) described 45 nonnative species (43 Pacific species including *Porphyra yezoensis* Ueda) that occurred at an active shellfish cultivation site in the Mediterranean Sea. By 2004 the list of exotic species at this cultivation site was increased to 56, including five nonnative species of *Grateloupia* C. Agardh (Verlaque et al. 2005). Outside the Mediterranean coast, other cases of nonnative species carried by this vector include *Sargassum muticum* (Ribera & Boudouresque 1995), *Codium fragile* (Suringar) Hariot subsp. *tomentosoides* (van Goor) P.C. Silva (Malinowski & Ramus 1973), and *Zostera japonica* Aschers and Graebn (Harrison & Bigley 1982).

Also contributing to the inadvertent spread of algal species worldwide is the modern seaweed industry. The farming of seaweeds has been a deliberate economic endeavor that has changed the distribution of many seaweeds. For example, the cultivation of *Porphyra* (nori) is the oldest and most advanced seaweed cultivation industry. The cultivation and harvesting of nori dates back hundreds of years among native peoples of both hemispheres (Miura & Aruga 1987; Turner 2003). In studying 11 of the 33 native Japanese species of *Porphyra* cultivated since the 1950's, Miura and Aruga (1987) showed that natural distribution patterns had been clearly affected by cultivation through transplantation of parent fronds and conchocelis. Some species ranges had been expanded while others were more narrowly circumscribed or even became endangered (Niwa et al. 2005). Griffin et al. (1999) reported the potential for similar disruptions to the distributions of endemic South African species of *Porphyra* due to harvesting pressures.

In addition to the exploitation of endemic species of marine algae, many non-native species of marine plants are being imported around the world. Siguan (2003) reported that 15 nonnative species of seaweeds (11 red algae, 4 brown algae) were presently being used for commercial and research purposes. Siguan (2003) raised several concerns regarding the accidental release of non-native species: 1) the lack of uniform international regulations on the management of introduction and transfer of marine plants within the seaweed industry; 2) research facilities with open seawater systems; 3) the uncontrolled commerce of exotic species by aquarium companies; and 4) fishing activities that

include the use of plants as packing material and the transport of algae-covered fishing nets.

CHAPTER II.

A MORPHOLOGICAL AND MOLECULAR INVESTIGATION OF THE *PORPHYRA 'PURPUREA'* COMPLEX IN THE NORTHWEST ATLANTIC

Abstract

Historically, *Porphyra purpurea* (Roth) Agardh has been the only monostromatic species recorded from the Northwest Atlantic that has a sectored male/female blade. Recently two additional monostromatic taxa with sectored blades have been reported from this geography. One is the newly described species *P. birdiae* Neefus et Mathieson and the other is *P. katadae* A. Miura, an introduced Asian species. In the present study, I use a combination of molecular, morphological, and ecological evaluations to characterize the identities, distributions, and relationships of the three taxa with sectored blades. Interspecific divergence in *rbc*L sequences ranged from 7.2% to 9.6%, while intraspecific variability in *rbc*L, ITS1, and SSU was very low. While *P. purpurea* has a broad distribution from northern Nova Scotia (Canada) to western Long Island Sound (New York, USA), *P. birdiae* occurs only north of Mount Desert Island (Maine, USA) and *P. katadae* is restricted to an area south of Cape Cod (Massachusetts, USA). The three species can be further distinguished by combinations of blade thickness, color, intertidal position, substratum, and seasonality.

In examining molecular variability in Northwest Atlantic *Porphyra purpurea* populations, several specimens were found to have *rbc*L sequences 100% identical to a GenBank accession for *P. rediviva,* while other specimens were 100% identical to the neotype of *P. purpurea*. Due to the molecular similarity in the *rbc*L sequence of both taxa (1 bp substitution), the holotype and isotypes of *P. rediviva* were sequenced. Comparisons of the *rbc*L and ITS1 sequences of the *P. rediviva* type specimens were 100% identical to the neotype and isoneotype of *P. purpurea.* Based upon these molecular findings and the failure of morphological and ecological features to clearly delineate the two taxa, *P. rediviva* is herein synonymized with *P. purpurea.*

Introduction

Despite a long history of domestication (Ueda 1932) and great economic value as a commercial crop (Mumford & Miura 1988, Yarish et al. 1998), the taxonomy of *Porphyra* remains problematic. Much confusion within this genus is attributable to the convergence and/or lack of divergence of morphological features during its long evolutionary history (Campbell 1980, Lindstrom & Cole 1993, Oliveira et al. 1995, Ragan et al. 1994, Stiller & Waaland 1993).

Early investigations of *Porphyra* (C. Agardh 1817, Kjellman 1883, Kützing 1843, Roth 1797) relied solely upon morphological characters for identification, including blade colour, size, shape, and texture, yielding species concepts that in many cases were too broad. Additional taxonomic features were later utilized, such as the number of cell layers (J. Agardh 1882), division formulae (Hus 1902), and chromosome numbers (Ishikawa 1921) that served to further delineate species, but have not completely eliminated taxonomic confusion within the genus. For example, division formulae have been shown to vary with culture conditions (Suto 1972) and different karyotypes have been reported within a "single" taxon (Holmes & Brodie 2004, Kapraun et al. 1991, Lindstrom & Cole 1992a, 1992b, Mitman & van der Meer 1994, Wilkes et al. 1999).

With the advent of molecular techniques, sequence data have proven to be extremely useful for delineating *Porphyra* species (Brodie et al. 1996, Broom et al. 2002, Farr et al. 2003, Klein et al. 2003). An assessment of variation in both nuclear and chloroplast encoded gene sequences has led to the

rearrangements of existing classifications, including the recognition of new species (Brodie et al. 1996, Brodie & Irvine 1997, Broom et al. 1999, Farr et al. 2003, Lindstrom & Fredericq 2003, Neefus et al. 2000, Neefus et al. 2002, Nelson et al. 2003, Stiller & Waaland 1996) and the synonymization of others (Broom et al. 2002). Sequence data from less constrained regions, such as the nuclear ribosomal internal transcribed spacer (ITS1), have also been useful for examining variation within individual species of *Porphyra* (Kunimoto et al. 1999a,1999b, Niwa et al. 2005).

Molecular tools have revealed unexpectedly large levels of diversity within geographies initially considered to have few species of *Porphyra* (Farr et al. 2003). Bird & McLachlan (1992), as well as Lindstrom & Cole (1992a), have suggested that some species of *Porphyra* in the Northwest Atlantic were too broadly defined and that the numbers of species underestimated. Based upon molecular and morphological taxonomic studies (Bird & McLachlan 1992, Broom et al. 2002, Coll & Cox 1977, Neefus et al. 2002, Neefus et al. submitted, Schneider & Searles 1991, West et al. 2005) eleven species are currently reported from the Northwest Atlantic: *P. amplissima* Kjellman, *P. birdiae* Neefus et Mathieson, *P. katadae* A. Miura, *P. linearis* Greville, *P. leucosticta* Thuret in LeJolis, *P. miniata* (C. Agardh) C. Agardh, *P. purpurea* (Roth) C. Agardh, *P. suborbiculata* Kjellman (= *P. carolinensis* Coll & Cox, cf. Broom et al. 2002, Coll and Cox 1977), *P. umbilicalis* Kutzing, *P. rosengurttii* Coll et Cox, and *P. yezoensis* Ueda.

One species in the Northwest Atlantic suspected of containing cryptic taxa is *Porphyra purpurea* (Bird & McLachlan 1992, Bray et al. 2006, Curtis 1997, Lindstrom & Cole 1992a, 1992b, 1993). Monostromatic thalli with male and female gametangia segregated on opposite, longitudinal "halves" were historically reported as *P. purpurea*. However, such a broad species concept has led to varying reports of morphology, chromosome counts, distribution, and seasonality (Bird & McLachlan 1992, Bray et al. 2006, Brodie & Irvine 2003, Curtis 1997, Lindstrom & Cole 1992a, Neefus et al. 2002, Wilkes et al. 1999). Sectored blade morphology is seen in several species worldwide (e.g. *P. brumalis* Mumford, *P. kurogii* S. C. Lindstrom, *P. aestivalis* S. C. Lindstrom et Fredericq) and because these species occur in three distinct clades of the *rbc*L gene trees, Lindstrom & Fredericq (2003) suggested that this morphological character (i.e. sectored blades) was homoplasious. Some of the taxonomic confusion within *Porphyra* in the Northwest Atlantic has been resolved with the recent description of a new species, *P. birdiae* (Neefus et al. 2002) and the report of an introduced Asian species *P. katadae* (Neefus et al. submitted) in southern New England. Both species have sexually segregated thalli similar to *P. purpurea*.

A very close relationship between *Porphyra purpurea* and the Pacific species *P. rediviva* was previously suggested by Lindstrom & Fredericq (2003), who found only a single bp difference between their *rbc*L sequences. Originally, Stiller and Waaland (1996) distinguished *P. rediviva* from *P. purpurea* based upon ecological and cytological differences and the occurrence of fixed rDNA

polymorphisms. That is, different restriction fragment length polymorphism (RFLP) patterns were reported in coding and intron regions of the nuclear small subunit ribosomal RNA (SSU) between Pacific *P. rediviva* and Atlantic *P. purpurea* (Stiller & Waaland 1996). However, Lindstrom & Fredericq (2003) concluded that the molecular difference was so small that further sampling of individuals and populations of *P. purpurea* and *P. rediviva* should be carried out. In the present study the relationship between *P. purpurea* and *P. rediviva* is critically examined including molecular assessments of their type specimens. In addition, a comparison of the morphology, ecology, and distribution of monostromatic *Porphyra* species with sectored blades in the Northwest Atlantic is included to provide reliable criteria for their distinction.

Materials and Methods

DNA Extraction, Sequence and Analysis

Collections of *Porphyra* specimens with sectored blades were made during low tides at multiple sites from Eastport, Maine to western Long Island Sound USA. Newly collected specimens were pressed on herbarium sheets, but before drying, a small sample (3 cm x 3 cm) was removed and preserved in 1/8" silica gel beads (Sigma ®) for DNA analysis. Voucher specimens for these collections have been deposited in the Albion R. Hodgdon Herbarium (NHA) at the University of New Hampshire (Appendix A). The holotype (WTU330639) and two isotypes (WTU330640, WTU330641) of *P. rediviva* from the University of Washington Herbarium (WTU), plus the neotype (BM000054930A) and an

isoneotype (BM000054930B) of *P. purpurea* from the Natural History Museum (BM) in London were examined and small samples (1 cm x 1 cm) were removed for DNA sequencing. Tissue samples (0.1-0.25 g) were ground in a mortar and pestle with autoclaved sand. Genomic DNA was extracted using a Puregene™ Isolation Kit per manufacturer's instructions.

Polymerase chain reactions (PCR) were performed in 50 µl volumes that contained 1-2 µl genomic DNA, 0.2 mM of each dNTP, 0.2 mM Mg^{2+}, 0.4 µl Taq DNA polymerase (5 μl^{-1}, Promega, Madison, Wis.), and 1X Magnesium Free Reaction Buffer B (Promega), with 0.4 µM of the amplification primers. A 1481 bp fragment, extending from position 67 (amino acid 23) of the large subunit of *rbc*L through the *rbc*L-*rbc*S intergenic spacer to the first codon of the small subunit, was amplified with primers F67 and rbc-spc (Teasdale et al. 2002). The rbc-spc primer is specific for the Bangiales and will not amplify DNA from common contaminant epiphytes. The *rbcL* gene was sequenced using primers described by Klein et al. (2003). Due to the unsuccessful amplification of the 1481 bp fragment from older, type specimens of *P. rediviva* (WTU330639, WTU330640, WTU330641) and *P. purpurea* (BM000054930A, BM000054930B), a shorter fragment (~595 bp) extending from position 952 of the large subunit of the *rbc*L into the *rbc*L-*rbc*S intergenic spacer was amplified with primers rbcL 7 (5'-TGTAAATGGATGCGTATGGC-3') and rbc-spc. Amplification of the nSSU rDNA coding region was preformed in two overlapping segments using four oligonucleotide primers: SSU 1F and E3R (Kunimoto et al. 2003), and G04 and J04 (Broom et al. 1999, Saunders & Kraft 1994). Additional internal sequencing

primers included G06 (Saunders & Kraft 1994). The ITS1-5.8S-ITS2 region was amplified using two primers: JBITS7 (Broom et al. 2002) and AB28 primers (Steane et al. 1991). The ITS1 region was sequenced using the JBITS7 and ITS1-R (5'-TATCCACCGTTAAGAG TTGTAT-3') primers. The corresponding amplification profiles are shown in Table 2.1. To confirm the size and decrease the presence of non-specific contaminants prior to sequencing, resulting PCR amplicons were gel-purified (Klein et al. 2003).

Amplified *rbc*L, SSU, and ITS1 products were sequenced on an ABI 373 Automated Sequencer at the University of New Hamphire's Hubbard Center for Genome Sciences using standard procedures as outlined by Germano and Klein (1999) and Klein et al. (2003). Sequences were edited in Chromas (version 2.2, Technelysium, Pty. Ltd., Tewantin, Queensland, Australia). Contiguous sequence assembly and alignments/comparisons were done in SeqMan II and MegAlign (version 6.1 for Windows, DNAStar, Inc., Madison, Wisconsin, USA), repectively. GenBank searches were completed using Blastn via the Net Search option in MegAlign.

Morphological Evaluations

Blade colour measurements were made at several positions within vegetative, male, and female regions of blades using an X-Rite Digital Swatchbook Reflective Spectrophotometer (Model DTP-22); the values from each region were averaged in Colorshop v.2.6.0 software (X-Rite, Grandville, Michigan, USA). Colour measurements are expressed in the Commission

Internationale d'Eclairage (CIE) L*a*b* tristimulus units (L* = lightness scale, a* = red/green scale, and b* = yellow/blue scale), which are based on a "standard observer" and are device-independent (Bunting 1998). Blade lengths, widths, and shapes were also recorded.

To quantify microscopic characters, measurements were taken from surface and transverse sections of vegetative and reproductive thalli using an Olympus BX40 microscope with a calibrated ocular micrometer. Vegetative sections were taken from the centre of the blade, well above the rhizoidal region. Male gametangial and zygotosporangial arrangements (Guiry 1990, Nelson et al. 1999) and packet sizes were also determined from surface and transverse sections. Female reproductive structures were distinguished by the presence of trichogynes or spindle-shaped cells including the first periclinal division (Holmes and Brodie 2004).

Subsets of several morphological characters (blade width and length; blade width to length ratio; vegetative, male, and female thalli thickness; vegetative cell height, width, and length; male and female gametangial packet height, width, and length; and L*a*b* colour of male, female, and vegetative blade regions) were subjected to discriminant analysis as well as multivariate and univariate ANOVA's to isolate characters or their combinations that would allow good separation between species. Discriminant analysis, ANOVA's and MANOVA's were completed in Systat® version 10.

Results

Molecular Characterization

Partial *rbc*L and *rbc*L-*rbc*S spacer sequences were obtained for 29 specimens from the Northwest Atlantic initially identified as *Porphyra purpurea*, plus one specimen from Ireland and another from France (Appendix A). The ITS1 region was sequenced from 12 Northwest Atlantic *P. purpurea* specimens, plus one from Ireland, and another from France. Among these specimens, *rbc*L varied by a single base pair substitution (G versus T at position 1299), while ITS1 varied by 1 or 2 substitutions. The 20 sequences with G in *rbc*L position 1299 were identical to partial *rbc*L (805 bp) and *rbc*L-*rbc*S spacer (71 bp) sequences from the neotype and isoneotype specimens of *P. purpurea* (BM00005454930A and BM00005454930B). The remaining 11 specimens (i.e. with T at position 1299) were 100% identical to an *rbc*L sequence by Lindstrom & Fredericq (2003) for a specimen identified as *P. rediviva* from the type location in Fildalgo Bay, Washington, USA (GenBank Accession #AF514280). Sequences from the *rbc*L gene of two Pacific specimens, initially identified as *P. rediviva*, from Yaquina Bay, Oregon, USA (GIH/HMSC1319, GIH/HMSC1658) were also identical to Lindstrom and Fredericq's sequence (Table 2.2). However, *rbc*L sequences of the holotype (WTU330639) and isotypes (WTU330640, WTU330641) of *P. rediviva* did not match Lindstrom and Fredericq's sequence, but rather were 100% identical to the *P. purpurea* neotype and isoneotype (Table 2.2). Additionally, the ITS1 sequences (210 bp) of the *P. purpurea* neotype and the *P. rediviva* holotype were 100% identical (Table 2.2). Among the 13 specimens

(i.e. 11 from New England and 2 from Oregon) matching Lindstrom and Fredericq's *rbc*L sequence, 6 individuals (NHA77975, NHA77885, NHA78002, NHA78039, GIH/HMSC1319, GIH/HMSC1658) shared identical ITS1 sequences with the *P. purpurea* neotype, while the others differed by one or two base pairs (Tables 2.2 & 2.3).

No intraspecific variation was found in the partial SSU coding sequences (~ 1768 bp) among specimens whose *rbc*L matched the *Porphyra purpurea* neotype. SSU sequences from New England and Oregon specimens with the alternate *rbc*L sequence (i.e. matching Lindstrom and Fredericq's sequence) differed from the *P. purpurea* neotype by a single base pair (exon position 863), with the exception of one New England specimen (NHA 77885) that was identical to the neotype.

In comparing partial *rbc*L sequences from *Porphyra purpurea* and *P. rediviva* with other sectored monostromatic species occurring in the Northwest Atlantic, *P. birdiae* was most divergent, differing by 9.6% from the *P. purpurea* and *P. rediviva* type specimens, and by 7.2% from *P. katadae* (Table 2.3). *Porphyra katadae* differed by 9.1% from both the *P. purpurea* and *P. rediviva* type specimens (Table 2.3).

Temporal, Geographical, and Ecological Patterns

Overall, *Porphyra purpurea* is an aseasonal annual occurring year round in the Northwest Atlantic, with reproductive individuals primarily occurring from late spring to late summer. Populations matching Lindstrom and Fredericq's *rbc*L

sequence were found during the winter and spring, with most collections (80%) occurring in March and April (Table 2.4 & Appendix A). Sixty percent of the specimens of this genotype were sexually reproductive, and of these, male gametangia were present on all specimens and a third had both male and female gametangia. Some thalli appeared to be non-sectored and entirely male (i.e. androdioecious), presumably originating from neutral spores produced on the male portion of the parent blade. Endosporangia (Guiry 1990) were present on a single specimen collected from Weymouth, Massachusetts.

Porphyra birdiae and *P. katadae* are seasonal annuals with the former occurring only during late summer and autumn, and the latter from winter through spring (Table 2.4 & Appendix A). Thus, while *P. birdiae* and *P. katadae* can be distinguished from each other by seasonality, neither can be separated from *P. purpurea* on this basis.

Northwest Atlantic populations matching the neotype of *Porphyra purpurea* occur from the Canadian Maritimes to Long Island Sound (Figure 2.1A). Specimens with the alternate *rbc*L sequence were collected from three sites in Maine, three in Massachusetts, and one in Connecticut (Figure 2.1A & Appendix A). The distribution of *P. birdiae* (Figure 2.1B) overlaps with the northerly portion of *P. purpurea*'s range, but it extends southward only to Mount Desert Island, Maine. *Porphya katadae* (Figure 2.1B) is more circumscribed and southerly than *P. birdiae*, occurring only in eastern Long Island Sound and southern Cape Cod Bay.

All three species occur within the intertidal to shallow subtidal (Table 2.4 & Appendix A), with the broadest ranges of elevation (high to low intertidal) displayed by both genotypes of *P. purpurea*. *Porphyra katadae* extends from the mid intertidal to shallow subtidal, while *P. birdiae* occurs in the mid to low intertidal. Populations matching the *P. purpurea* neotype were found from the open coast to sheltered embayments and estuarine tidal rapids, while those with the alternate *rbc*L sequence were found almost exclusively in sheltered embayments. While *P. birdiae* occurs on exposed to moderately exposed open coasts, *P. katadae* is restricted to sheltered shallow embayments and tidal rapids. Substratum preferences varied. Specimens of *Porphyra purpurea* matching the neotype *rbc*L sequence were mostly epilithic, while those with the alternate sequence were commonly found on small stones or as an epiphyte on *Fucus vesiculosus* L. *Porphyra birdiae* appears to be exclusively epilithic, while *P. katadae* is always epiphytic.

Morphological and Anatomical Characterization

Table 2.4 summarizes morphological, anatomical and ecological features of sectored, monostromatic *Porphyra* species occurring in the Northwest Atlantic. *Porphyra purpurea* specimens matching the neotype *rbc*L sequence are compared to those with the alternate sequence. The same features are also compared to the original description of *P. rediviva* from the Pacific by Stiller & Waaland (1996) and to descriptions of *P. birdiae,* and *P. katadae* detailed in previous studies (Neefus et al. 2002, Neefus et al. submitted).

The blade shape of Northwest Atlantic *Porphyra purpurea* with the alternate *rbc*L sequence is often irregular, but occasionally ovate or bilobate (butterfly-shaped) and averages 10.97 cm (±8.63SD) in length to 8.63 cm (±5.94SD) in width. Its margins are sparsely ruffled and rarely laciniate, while the base is cordate to pseudoumbilicate. In reproductive individuals, male and female gametangia occur on opposite, longitudinally divided "halves" of the blade. Vegetative portions of the thalli range in colour from greenish-brown to bronze (average CIE L:62.43, a:1.51, b:37.87), with male portions fading in time to a yellow-tan (average CIE L:80.8, a:-2.12, b:34.98) and female portions changing to a deep rust-red (average CIE L:55.97, a:13.65, b:48.39). Vegetative thalli range in thickness from 27-61 µm (mean 46.4 ±11.4SD); male portions vary from 30-69 µm (mean 51.0 ±12.2SD), while female sections are 39.5-41.9 µm (mean 40.7 ±1.76SD). Vegetative cell dimensions are 7-17 µm x 10-24 µm in surface view and 15-30 µm tall in transverse view; they have a single stellate chloroplast. Male gametangial packets are 12-24 µm x 17-30 µm in surface view occur as 8 tiers of 8, while zygotosporangial packets are 19.7-22.2 µm x 24.7-32.1 µm and are arranged as 4 tiers of 4. Blade dimensions of the holotype (WTU 330639) and isotypes (WTU 330641, WTU 330642) of *Porphyra rediviva* average 26.8 cm long x 13 cm wide. The average vegetative thallus thickness of both isotypes is 47 µm (Table 2.4).

Discriminate analysis separated *Porphyra katadae* from both genotypes of *P. purpurea* and *P. birdiae* based upon combinations of morphological characters. Univariate analysis (ANOVA) showed that the vegetative thallus

colour of *P. katadae* was lighter ($P \leq .00001$) than *P. purpurea* or *P. birdiae* (Figure 2.2). Both vegetative and male portions of *P. katadae* blades were thinner ($P \leq .002$) and had smaller vegetative cell dimensions ($P \leq .03$) than the *P. purpurea* genotype and *P. birdiae.*

There is a significant overlap in the ranges of vegetative and reproductive thallus thicknesses among the two *Porphyra purpurea* genotypes, *P. katadae,* and the original description of *P. rediviva* (Table 2.4). All taxa are monostromatic and their cells contain a single stellate chloroplast. Male gametangial cells occur in packets that are eight tiers high in both *P. purpurea* genotypes, *P. rediviva* and *P. birdiae*, but are only four tiers high in *P. katadae.* Zygotosporantial packets of *P. katadae* are two tiers high versus four in both *P. purpurea* genotypes, *P. rediviva*, and *P. birdiae.*

Discussion

Synonymization of *Porphyra purpurea* and *Porphyra rediviva*

Morphological, ecological, and cytological characters in the original description of *Porphyra rediviva* fall within the ranges found in *P. purpurea* from the Northwest Atlantic. Stiller and Waaland (1996) reported that seasonality and habitat differences separated Pacific *P. rediviva* from Atlantic *P. purpurea* and that *P. rediviva* occurred (in drift) year round. Since *P. purpurea* in the Northwest Atlantic is an aseasonal annual, seasonality does not appear to distinguish these species (Table 2.4). Although Stiller and Waaland (1996) describe *P. rediviva* as occurring exclusively in salt marshes, *Porphyra. purpurea* in the Northwest

Atlantic occurs in a broad range of habitats, including diverse estuarine environments (Bray et al. 2006, Neefus et al. 2002). Additionally, the zonation patterns and substratum preferences of Northwest Atlantic *P. purpurea* are similar to those originally described for *P. rediviva* (Table 2.4). Although Stiller and Waaland (1996) reported differences in chromosome numbers between *P. rediviva* (n = 4) and *P. purpurea* (n = 5), reports of chromosome number for Atlantic *P. purpurea* vary (Lindstrom and Cole 1992a, 1993, Wilkes et al. 1999). Persistence of pigmented chloroplasts during spermatangial divisions can make the observation of chromosomes difficult and counts suspect (Neefus et al. 2002). Holmes & Brodie (2004) reported 3 to 7 chromosomes in different cells of the same blade of *P. dioica.* Counts of 3, 4 and 5 chromosomes were routinely observed in *P. mumfordii* S.C. Lindstrom & K.M. Cole in different cells of the same thallus (S. C. Lindstrom pers. comm.). Inconsistent reports of chromosome numbers may also result from inaccurate species identification (Kapraun et al. 1991, Mitman & van der Meer 1994, Wilkes et al. 1999).

While the partial *rbc*L sequences of holotype and isotype specimens of *Porphyra rediviva*, plus the neotype and isoneotype of *P. purpurea* are all identical, the one base pair substitution of some specimens, including Lindstrom and Fredericq's *P. 'rediviva'* sequence, fall within the range of reported intraspecific variation for some *Porphyra* species (Brodie et al. 1998, Klein et al. 2003, Lindstrom & Fredericq 2003). Although the chloroplast *rbc*L gene has been shown to be less variable within *Porphyra* species than the multi-copied, nuclear SSU gene sequences (Klein et al. 2003), some intraspecific variations does

occur. Klein et al. (2003) reported variations (1 bp substitution) in the *rbc*L sequence among New Hampshire populations of *P. umbilicalis.* Brodie et al. (1998) concluded that in the absence of any discernible morphological differences, a single transition in the *rbc*L region between *P. purpurea* from multiple sites did not warrant taxonomic distinction.

The ITS1 region is a highly variable region in *Porphyra* that has been widely used in intraspecific studies (Broom et al. 2002, Kunimoto et al. 1999a, 1999b, Mizukami et al. 1999, Niwa & Aruga 2003, Niwa et al. 2004, 2005). Stiller & Waaland (1993) suggested that the ITS region provided an alternative region of greater sequence variability than the more conserved SSU gene. The ITS1 sequences of the holotype and isotype of *P. rediviva* were identical to the neotype of *P. purpurea*, while varying only slightly (1-2 bp) from some other *P. purpurea* specimens (Table 2.2). Similarly, the ITS1 sequences of Northwest Atlantic specimens with the alternate *rbc*L sequence were identical to some *P. purpurea* specimens with the neotype *rbc*L sequence, while varying no more than 2 bp from others (Table 2.2). Kunimoto et al. (1999a) and Mizukami et al. (1999) found greater levels of intraspecific variation within the ITS1 region of *P. yezoensis* than we found in the present study. Likewise, Broom et al. (2002) argued for the synonymization of *P. carolinensis* and *P. lilliputiana* W.A. Nelson, G.A. Knight et M.W. Hawkes with *P. suborbiculata,* despite the presence of greater divergence in the ITS1 region than exists among *P. purpurea* and the type specimen of *P. rediviva*.

Stiller & Waaland (1996), who used restriction fragment length polymorphism (RFLP) patterns, distinguished *Porphyra rediviva* from *P. purpurea* based in part upon the presence of "fixed" SSU rDNA polymorphisms. They reported four polymorphisms between *P. rediviva* and Atlantic *P. purpurea,* two in the coding region and two within the intron. Of the Atlantic *P. purpurea* specimens used by Stiller & Waaland (1996), they stated that the group I intron near the 3' end was present in most British specimens, absent in the Maine populations, and mixed in Nova Scotia populations. They attributed such differences in intron distribution to genetic isolation or restricted gene flow among geographically distinct population. However, Teasdale (2004) argued that SSU introns may exist in every *Porphyra* individual, but their detection can be hindered by factors such as short allele dominance and a lack of a perfect match between the primer and template. He showed that intron detection increased by >75% for *P. umbilicalis* and >45% for *P. amplissima* when combining an intron-internal primer and an external primer as compared to using only flanking primers. Furthermore, in a survey of the helix 50 group-I intron using the more specific internal primer, Teasdale (2004) concluded that all *P. umbilicalis* individuals across a large geographic range had introns and that some individuals had more than one size variant of the helix 50 intron. He stated that the occurrence of multiple forms of introns within an individual may be a common occurrence and that gene conversion mechanisms may not be homogenizing all rDNA cistrons. Under such conditions "fixed polymorphisms" within introns would be difficult to confirm within species, populations, and even individuals.

While SSU divergence has been shown to be very high among *Porphyra* species (Kunimoto et al. 1999a, Oliveira et al. 1995, Ragan et al. 1994), some intraspecific variation also occurs (Broom et al. 2002, Klein et al. 2003, Müller et al. 2001). In studying Northwest Atlantic taxa of *Porphyra*, Klein et al. (2003) reported intraspecific SSU sequence variation of 0.0-0.34% among eight individuals of *P. umbilicalis,* 0.0-0.9% for ten individuals of *P. purpurea,* 0.0-2.1% in 12 samples of *P. amplissima,* and 0-3.5% for eight samples of *P. linearis.* Klein et al. (2003) reported some intraspecific variation among specimens collected from different locations and seasons, but in other collections, sequence differences were observed among individuals collected at the same time and place. Within the partial coding region sequenced in this study, only a single polymorphic SSU site was detected among individuals of the two *rbc*L genotypes of *P. purpurea.*

Stiller & Waaland (1996) reported no intraspecific variability in riboprints of the SSU gene and little apparent variability in the less conserved ITS1 regions of *Porphyra rediviva* across a wide geographical range (northern California to northern Washington). We have confirmed their findings, as only 1 polymorphic site was found in the *rbc*L and SSU and no more than 2 base pair substitutions were found in the ITS1 regions within and between populations of *P. purpurea* from the Atlantic and *P. rediviva* from the Pacific Oceans. Additionally, no differences were found in the *rbc*L or ITS1 regions sequences between the type specimens of *P. purpurea* and *P. rediviva.*

As noted above, the descriptions of *Porphyra purpurea* and *P. rediviva* are remarkably similar (Table 2.3). Thus, it is concluded that a failure to delineate between morphology, seasonality, and habitat of the two taxa, plus the occurrence of identical *rbc*L and ITS1 sequences in the type specimens support the conspecificity of *P. purpurea* and *P. rediviva*. As *P. purpurea* (Roth) C. Agardh (1817) is the oldest name, *P. rediviva* Stiller et Waaland (1996) is herewith synonymized with *P. purpurea.* Furthermore, the single base pair substitution in some Atlantic and Pacific *rbc*L sequences, including Lindstrom and Fredericq's Fidalgo Bay specimen, is considered intraspecific variation of no taxonomic significance.

Porphyra purpurea is not the only *Porphyra* species that occurs in both the Atlantic and Pacific Oceans. Broom et al. (2002) reported a broad distribution of *P. suborbiculata* in multiple oceans. The similarities in ITS1 region sequences between Atlantic and Pacific populations of *P. purpurea* do not support prolonged genetic separation and show a closer relationship than the sibling species pairs identified by Lindstrom & Fredericq (2003). While in some cases the lack of sequence variability in the ITS1 has been used as evidence of recent introductions (Neefus et al. submitted), the lack of variability in both Atlantic and Pacific populations appears to be equal, making the designation of a parent population less clear.

Comparisons of *Porphyra purpurea*, *P. birdiae*, and *P. katadae*

While the Northwest Atlantic distribution of *Porphyra purpurea* overlaps with both *P. birdiae* and *P. katadae*, the southernmost occurrence of *P. birdiae* (Mount Desert Island, Maine) and northernmost occurrence of *P. katadae* (Cape Cod Bay, Massachusetts) are widely separated (Figure 2.1A). The ecologies of *P. birdiae* and *P. katadae* also differ, with the former occurring mainly in exposed areas, while the latter is restricted to shallow embayments and tidal rapids (Table 2.4). By contrast, *P. purpurea* occurs in a broad range of habitats from exposed open coastal to estuarine (Bray et al. 2006).

Zonation patterns of the three different taxa also overlap (Table 2.4); however, only *Porphyra purpurea* extends into the high intertidal, while *P. birdiae* is more common in the low intertidal, and *P. katadae* occurs in the shallow subtidal. Additionally, *P. katadae* has been found only as an epiphyte, while *P. purpurea* and *P. birdiae* are frequently epilithic. With respect to seasonality, *P. purpurea* is an aseasonal annual with maximum occurrence in spring-summer, while *P. birdiae* occurs in summer-autumn versus the winter-spring occurrence of *P. katadae.*

The most pronounced morphological difference among *Porphyra purpurea, P. birdiae* and *P. katadae* occurs in thallus thickness (Table 2.4). Neefus et al. (2002) distinguished *P. birdiae* from *P. purpurea* based in part upon its thallus thickness and distinct wide, pale blade margins. In this study discriminate analysis of morphological characters separated *P. katadae* from all

other taxa under consideration; most significantly, the thalli of *P. katadae* tend to be thinner, more ruffled, and lighter in colour than *P. purpurea* or *P. birdiae.*

Although no single morphological or ecological character can clearly separate the species, molecular data show no ambiguities in delineating *P. purpurea, P. birdiae,* and *P. katadae*. Divergence of 7.2%-9.6% occurs among the three taxa based upon *rbc*L sequences (Table 2.3). In both parsimony and neighbor-joining trees based upon *rbc*L sequences, *P. purpurea* and *P. birdiae* occur in separate clades (Lindstrom & Fredericq 2003). Klein et al. (2003) showed distance phylograms from partial SSU and *rbc*L gene sequences that also place *P. purpurea* and *P. birdiae* in separate clades.

Conclusions

While intraspecific variation was shown to exist in DNA sequences of *Porphyra purpurea*, its molecular delineation from other Northwest Atlantic monostromatic *Porphyra* with sectored blades (e.g. *P. birdiae*, *P. katadae*) is unequivocal. The use of DNA sequencing has provided a reliable tool to resolve the identities, distributions, and relationships of these morphologically similar species as well as a strong argument for the synonymization of *P. purpurea* and *P. rediviva.*

While this study focused upon a morphological subset of *Porphyra* taxa occurring in the Northwest Atlantic, the presence of additional taxa with sectored monostromatic blades cannot be ruled out. The lack of comprehensive studies of native *Porphyra* worldwide and the ever increasing speed and range of

commercial shipping greatly enhances the possibility of introductions and re-introductions (Fofonoff et al. 2003, Siguan 2003).

Table 2.1. Amplification profiles.

Regions	Primers	Anneal	Extension	Denature	Cycles
rbc L	F67 and rbc-spc	45°C, 1 min	72°C, 90 s	93°C, 1min	30
ITS1	Jbits7 and AB28	60°C, 1 min	72°C, 90 s	94°C, 30s	30
SSU	G04 and J04	54°C, 1 min	72°C, 45 s	93°C, 30s	30
SSU	SSU1F and E3R	55.6°C, 1 min	72°C, 45 s	93°C, 30s	30

All reactions heated intially to 93°C or 94°C for 3 minutes to denature templates prior to the first round of amplification. A final cycle was carried out with an extension time of 10 minutes after 30 cycles of amplification.

Table 2.2. Base pair substitutions in partial *rbc* L and ITS1 sequences of the *Porphyra purpurea* neotype, the *P. rediviva* holotype, the *P. purpurea rbc* L genotype matching the neotype, and the alternate *P. purpurea rbc* L genotype. Diagonal line and below = ITS1 (210 bp); above diagonal line = *rbc* L (1358 bp, position 109-1467) plus partial rbcL-rbcS spacer (72 bp).

		***rbc* L**			
		P. purpurea neotype[1] BM000054930A	*P. rediviva* holotype[2] WTU330639	*P. purpurea* with neotype *rbc* L (n = 18)	*P. purpurea* with alternate *rbc* L *(n = 15)*
ITS1	*P. purpurea* neotype[1] BM000054930A	0	0	0	1
	P. rediviva holotype[2] WTU330639	0	0	0	1
	P. purpurea with neotype *rbc* L (n = 18)	0-2	0-2	0-2	1
	P. purpurea with alternate *rbcL* (n = 15)	0-2	0-1	0-2	0

[1] *P. purpurea* neotype = 805 bp *rbc* L plus 71 bp rbcL-rbcS spacer.

[2] *P. rediviva* holotype = 752 bp *rbc* L.

Table 2.3. Percent divergence in partial *rbc* L (1358 bp, position 109-1467) plus partial *rbc* L-*rbc* S spacer (72 bp) of *Porphyra purpurea*, *P. katadae*, and *P. birdiae*.

		1	2	3
Porphyra purpurea	1	*		
P. katadae	2	9.1	*	
P. birdiae	3	9.6	7.2	*

Table 2.4. A comparison of taxonomic characters among *Porphyra purpurea* , *P. purpurea* [1] with the alternate *rbc* L sequence, *P. rediviva* , *P. birdiae* and *P. katadae.*

	Porphyra purpurea Bray *et al. 2006*	***Porphyra purpurea*** [1] with alternate *rbc* L sequence This study	***Porphyra rediviva*** Stiller & Waaland 1996	***Porphyra birdiae*** Neefus *et al.* 2002	***Poprhyra katadae*** Neefus *et al.* submitted
Blade Morphology					
Color	Light mauve (CIE L:63.92, a:6.62, b:14.94) in vegetative area; tan (CIE L:83.55, a:1.06, b:18.2) in male portion and dark mauve to red (CIE L:50.45, a:20.59, b:10.1) in female portion.	Greenish-brown to bronze (CIE L:62.43, a:1.51, b:37.87) in vegetative area; yellow to light tan (CIE L:80.8, a:-2.12, b:34.98) in male portion and rust-red (CIE L:55.97, a:13.65, b:48.39) in female portion.	Yellow-brown to red-brown, drift thalli; pale yellow to pale greenish-yellow to tawny-brown to pale purple-gray to rosey-purple.	Greenish-brown to chocolate-brown (CIE L:39.95, a:3.02,b:24.58) and lighter near holdfast (CIE L:54.62, a:2.14, b:21:28); male portion yellow-green (CIE L:78.16, a:-1.22, b:31.53) female portion reddish brown (CIE L:55.54, a:10.86, b:33.68)	Reddish-brown (CIE L:79.34, a:2.61, b:22.19) in vegetative area; pale yellow (CIE L: 88.54, a:-.33, b:17.7) in male portion and purplish-red (CIE L:78.36, a:5.24, b:20.17) in female portion.
Shape	Ranging from orbiculate to fusiform, with tips mostly attenuate.	Irregular, occasionally ovate or bilobate (butterfly-shape).	Thalli irregular, often lobed.	Commonly irregular, occasionally ovate, bilobate (butterfly-shape) or trilobate, rarely wedge shaped or elongate.	Round, ovate, lanceolate, elongate; falcate with cordate.
Margin	Moderately ruffled and laciniate; entire to slightly serrate.	Slightly ruffled; entire; occasionally laciniate.	n/a	Frequently very laciniate, with moderate to sparse ruffles not normally extending to blade center; rarely with crenulate (frilly) portions.	Entire, ruffled in lacinate specimens.
Base	Stipitate to cordate.	Moderately to deeply cordate; pseudo-umbilicate.	n/a	Pseudo-umbilicate.	Cordate, pseudo-umbilicate.
Dimensions	4-41.5 cm (mean 22.3 ±8.73SD) long x 6-28.5 (mean 11.2 ±6.24SD) wide.	3-24.5cm (mean 10.97 ±8.63SD) long x 2-24 cm (mean 8.63 ±5.94SD) wide.	up to 70 cm long x 40 cm wide (Average for holotype and isotype is 26.8 cm long x 13 cm wide)	5-21 cm long x 5-27cm wide	4.5-20.5 cm (mean 12.88 ±4.98SD) long x 1-14.5 cm (mean 8.15 ±5.04SD) wide.
Cell Morphology					
Vegetative					
Thallus Thickness	24-69 µm (mean 42.6 ±9.4SD)	27-61 µm (mean 46.4 ±11.4SD)	60-75 µm 30-45 µm (drift material)	50-75 µm	15-37 µm (mean 29.9 ±7.34)
Cell Layers	one	one	one	one	one
Chloroplast	Single; stellate	Single; stellate	Single; stellate	Single; stellate	Single; stellate
Cell Dimensions	5-35 µm x 5-20 µm in surface view; 10-40 µm tall in transverse view.	7-17 µm x 10-24 µm in surface view; 15-30 µm tall in transverse view.	20-55µm highx10-20µmwide	12-17 µm x 17-30 µm in surface view; 30-35 µm tall in transverse view.	7-17 µm x 7-22 µm in surface view; 7-25 µm tall in transverse view.

Table 2.4. Continued.

	Porphyra purpurea Bray *et al. 2006*	***Porphyra purpurea*** [1] with alternate *rbc*L sequence This study	***Porphyra rediviva*** Stiller & Waaland 1996	***Porphyra birdiae*** Neefus *et al.* 2002	***Poprhyra katadae*** Neefus *et al.* submitted
Male Gamatangia					
Thallus Thickness	24-64 µm (mean 43.9 ±11SD)	30-69 µm (mean 51 ±12.2SD)	n/a	58-75 µm	20-32 µm (mean 26.3 ±2.9SD)
Arrangement	8 tiers of 4 (or 8)	8 tiers of 8	tiers of 8 (or 16)	8 tiers of 8 (or 16)	4 tiers of 8 (or 16)
Packet Dimensions	9.8-44.5 µm x 5-32 µm in surface view; 17.3-49.4 µm tall in transverse view.	12-24 µm x 17-30 µm in surface view; 24-44 µm tall in transverse view.	n/a	20-30 µm x 24-35 µm in surface view.	12-20 µm x 23-30 µm in surface view; 16-30 µm tall in transverse view.
Cell Dimensions	2.1-7 µm x 2.1-9 µm in surface view; 2.1-7 µm tall in transverse view.	3.7-4.9 µm x4.9-8.6 µm in surface view; 2.4-4.9 µm tall in transverse view.	n/a	4.0-5.0 µm in diameter	2.4-6.1 µm x 3.7-6.1 µm in surface view; 2.4-4.9 µm tall in transverse view.
Zygotosporangia					
Thallus Thickness	34.6-81.5 µm (mean 55.8 ±13SD)	39.5-41.9 µm (mean40.7 ±1.76SD)	n/a	58-95 µm	27.1-37 µm (mean 30.8 ±4.7SD)
Arrangement	2 (or 4) tiers of 4	2 (or 4) tiers of 4	tiers of 4 (or 8)	4 tiers of 4	2 tiers of 4
Packet Dimensions	14.8-29.6 µm x 17.3-37.1 µm in surface view; 19.8-58.1 µm tall in transverse view.	19.7-22.2 µm x 24.7-32.1 µm in surface view; 24.7-34.5 µm tall in transverse view.	n/a	20-25 µm x 20-30 µm in surface view.	17.2-24.7 µm x 18.5-29.6 µm in surface view; 19.7-34.5 µm tall in transverse view.
Cell Dimensions	5-15 µm x 5-17.5µm in surface view;7.6-22.5 µm tall in transverse view.	8.6-9.8 µm x 12.3-14.8 µm in surface view; 9.8-14.8µm tall in transverse view.	n/a	8-12 µm in diameter	7.4-12.3 µm x 8.6-22.3 µm in surface view; 9.8-14.8 µm tall in transverse view.
Ecology					
Seasonality	Aseasonal annual with maximum occurrence in summer.	Winter-spring.	Late summer-spring; drift material collected year round	Summer-autumn annual	Winter-spring.
Elevation	High to low littoral.	Mid to low littoral.	High littoral.	Mid-littoral.	Mid to shallow sublittoral.
Substrata	Epilithic on boulders and pebbles; Epizoic on barnacles and mollusks; Epiphytic	Epiphytic on *Fucus vesiculosus* ; Epilithic on small stones.	Reproductive fronds on rocks and stones; nonreproductive fronds in drift or tangled in salt marsh plants.	Epilithic.	Epiphytic on *Gracilaria tikvahiae, Chondrus crispus,* and *Dumontia contorta.*
Habitat	Open coast to estuarine tidal rapids.	Semi-exposed open coast to estuarine.	Brackish marshes.	Exposed to moderately exposed open coasts.	Shallow embayments, tidal rapids.

[1] *P. purpurea* specimens with *rbc*L sequences that differ from the neotype by a single G to T transition at position 1299.

Figure 2.1. Northwest Atlantic distributional patterns of monostromatic *Porphyra* taxa with sectored blades. A) Distributions of the two *rbc*L genotypes of *Porphyra purpurea*; solid squares represent populations matching the *P. purpurea* neotype sequence, while open squares indicate the alternate genotype with a single base substitution. B) Distributions of *P. birdiae* (solid circles) and *P. katadae* (solid diamonds).

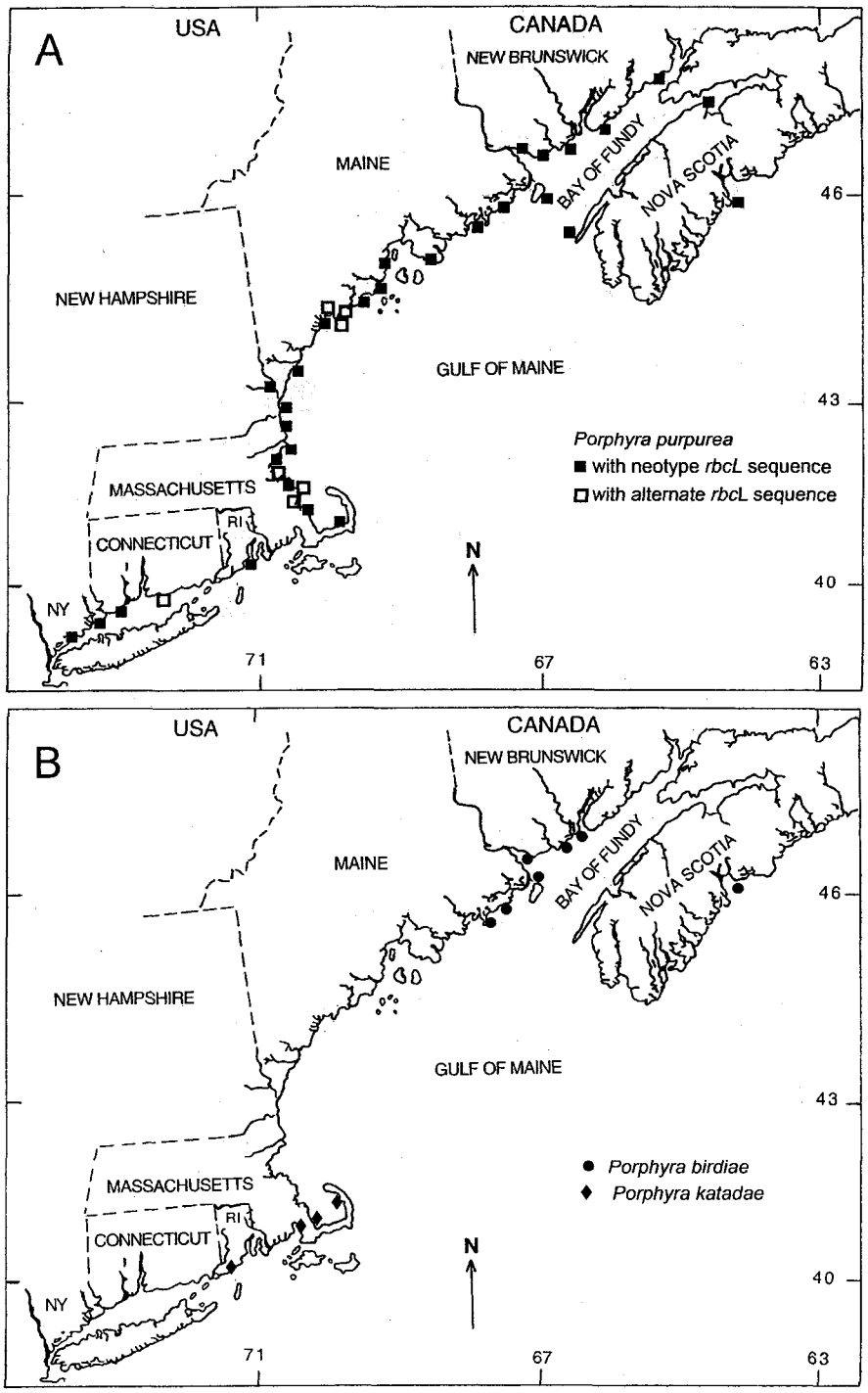
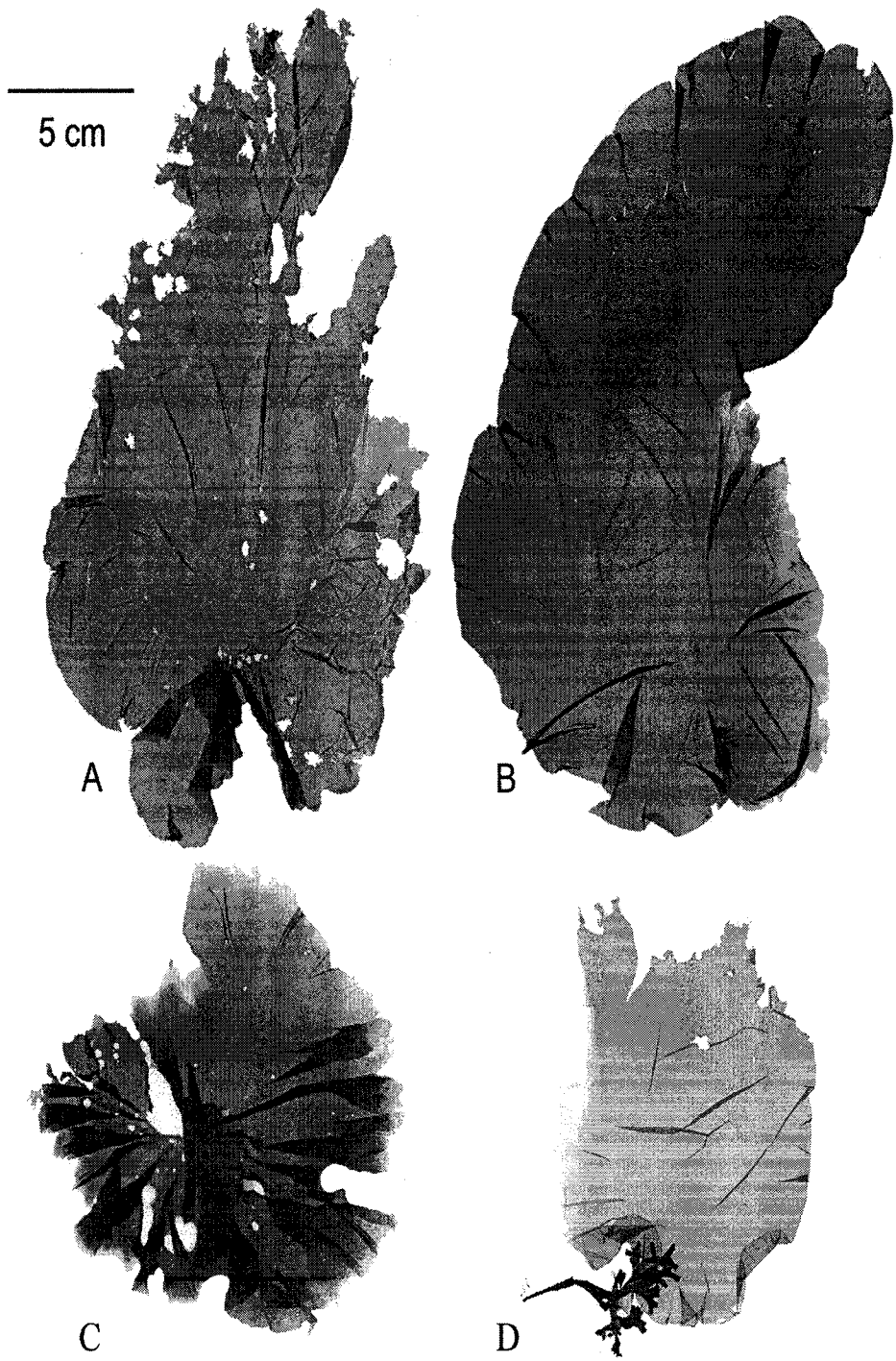


Figure 2.2. Blade morphology and color of A) *Porphyra purpurea* with rbcL matching the neotype, B) *P. purpurea* with the alternate rbcL sequence, C) *P. birdiae*, and D) *P. katadae*



CHAPTER III

THE OCCURRENCE OF INTRODUCED ASIATIC SPECIES OF *PORPHYRA* TO THE COAST OF NEW ENGLAND AND GULF COAST OF TEXAS

Abstract

Porphyra yezoensis Udea was first described from northeastern Honshu and southwestern Hokkaido, Japan. It is one of eleven cultivated species of nori in Japan. It has also been reported from China and East Russia. Based upon comparisons of sequences from the chloroplast *ribulose bisphosphate carboxylase oxygenase large subunit* (*rbc*L) gene and the nuclear ribosomal DNA internal transcribed spacer region (ITS1), three strains of *P. yezoensis* are reported herein from the New England and Texas coast. Based upon both *rbc*L and ITS1 sequences, the New England and Gulf Coast populations shared a single common strain (f. *yezoensis*). However, comparisons of ITS1 sequences revealed that the second New England strain had identical sequences to the commercial strain, f. *narawaensis*, while the second Texas strain exhibited sequences with closer affinities to Chinese specimens than Japanese populations of f. *yezoensis*. A second Asiatic species, *Porphyra katadae*, was also discovered in New England based upon sequence data from the *rbc*L gene and ITS1 region. The occurrence, distribution, molecular and morphological

features are reported from New England and three sites in Texas of these Asiatic *Porphyra* species.

Introduction

The red alga *Porphyra* (C. Agardh) C. Agardh is a fast-growing annual seaweed with a biphasic life history that alternates between a haploid gametophytic blade and a diploid microscopic filamentous sporophyte or "conchocelis" stage (Brodie & Irvine 2003). Blades occur either seasonally or aseasonally, are monoecious or dioecious, 1-2 cells thick, and grow attached to rocks, shellfish, or other seaweeds within the intertidal or shallow subtidal of temperate regions. Although *Porphyra* has been cultivated for food (nori) in Asia for 300 years, its production has increased greatly during the last 50 years due to the development of floating net systems, synthetic fiber nets, and the ability to "seed" nets with spores from cultured conchocelis (Miura & Aruga 1987). Annual global aquaculture production of *Porphyra* exceeds 1 million metric tonnes (FAO 2003).

Porphyra yezoensis Ueda, which is one of eleven native Asian species cultivated in Japan (Miura 1988), was first described from northeastern Honshu and southwestern Hokkaido, Japan (Ueda 1932). It also occurs along the coast of China (Tseng 1984) and eastern Russia (Perestenko 1994), where it grows on rocks or other seaweeds within the low intertidal and shallow subtidal zones from autumn through spring (Miura 1988; Miura & Aruga 1987). *Porphyra yezoensis* is cultivated primarily to produce "Hoshi-nori", which are rectangular paper-like dried sheets used to make sushi (Miura 1988; Miura & Aruga 1987). Although numerous cultivars of *P. yezoensis* are grown in Japan, most of them have been

developed from a single strain isolated during the late 1960's from a nori farm at Narawa in the Chiba Prefecture (Kunimoto *et al.* 1999a; Miura 1984; Niwa & Aruga 2003). The strain is fast growing, remains vegetative for a relatively long time, and produces high quality nori sheets (Miura 1984). This strain can be morphologically distinguished from wild *P. yezoensis* by its longer (up to 1 m), narrower blades, and it has been designated *P. yezoensis* forma *narawaensis* Miura. By the late 1980's nori cultivation along the Japanese coast was so extensive that Miura and Aruga (1987) judged it to be at a "nearly saturated level", with practically all production based upon cultivars of *P. yezoensis* f. *narawaensis*. Extensive populations of f. *narawaensis* have become established on natural substrata in areas of cultivation, resulting in the displacement and/or extinction of other native *Porphyra* species, a decrease in genetic diversity, and the alteration of community structure and ecology of the Japanese coast (Miura & Aruga 1987).

Several molecular studies have examined divergence within and between cultivated and wild strains of *Porphyra yezoensis* as well as other Japanese *Porphyra* species (Kunimoto *et al.* 1999a; Mizukami *et al.* 1999; Niwa & Aruga 2003). Exon regions of SSU rRNA gene differ between *Porphyra* species, but are identical within all strains of *P. yezoensis* (Kunimoto *et al.* 1999a). By contrast, sequences of the ITS1 spacer vary within species and Kunimoto *et al.* (1999a) reports up to 3% divergence among individuals of *P. yezoensis* in samples from a single population from Nanahaema, Hakodate, Hokkaido. Although the ITS1 region of *P. yezoensis* f. *narawaensis* differs from wild strains

by up to 4%, there is no variation within more than a dozen cultivars of f. *narawaensis* (Kunimoto *et al.* 1999a; Mizukami *et al.* 2003; Niwa & Aruga 2003). Using AFLP analysis, Niwa *et al.* (2004) found extremely low levels of genetic variation between two of these cultivars.

A second Asian species, *Porphyra katadae* Miura, which was originally described from Japan, has a continuous distribution on Hokkaido, but is restricted to estuaries on Honshu and Kyushu (Miura 1968). The species has also been recorded from Korea (Miura 1968), the eastern coast of Russia (Perestenko 1994), and Qingdoa, China (Tseng & Chang 1978). In Japan, the blades grow within the intertidal, shallow subtidal and tide pools on rocks, mussels, or other seaweeds [e.g. *Grateloupia filicina* (J.V. Lamouroux) C. Agardh] from January through March (Miura 1968). *Porphyra katadae* is not actively cultivated in Japan, but in areas where abundant natural populations occur, it is collected to make Hoshi-nori (Miura 1988). No commercial cultivars of *P. katadae* have been described and only limited molecular information is available. Sequence for the SSU rRNA gene, ITS1 spacer, and *rbc*L are available on GenBank (Kunimoto AB013184 & AB017090, Kito AB118583) for specimens from Kawatana, Yamaguchi, Japan. Chinese specimens, which have thicker blades than Japanese plants, were described by Tseng & Chang (1978) as var. *hemiphylla*. One sequence spanning the ITS1, 5.8s and ITS2 regions is available on GenBank (Hu, He, & Duan AY368577) for a specimen identified as *P. katatdae* var. *hemiphylla* from Qingdao, China.

To date, there have been only a few documented cases of introduced *Porphyra* species. Kornmann (1986) reported a non-indigenous population of *P. yezoensis* on the island of Helgoland, Germany, which he identified based upon culture characteristics. However, his identification could not be confirmed by Brodie and Irvine (2003) who sequenced the *rbc*L-*rbc*S spacer of his culture and found that it differed from a Japanese *P. yezoensis* specimen. Verlaque (2001) listed *P. yezoensis* as being inadvertently introduced to the Thau Lagoon (Hérault, France) via shellfish aquaculture.

During the 1990's, a small aquaculture company, Coastal Plantations, began growing two Japanese strains (U51 and H25) of *Porphyra yezoensis* f. *narawaensis* Miura in Cobscook Bay near Eastport, Maine USA (Levine 1998). Aquaculture permits were approved based upon evidence that the combination of photoperiod and temperature would not allow sexual reproduction. Extensive surveys of the "grow-out" sites found some escaped individuals during the growing season, but no established (over-wintering) populations (Watson et al. 1998, 1999). More recently, West et al. (2005) confirmed the occurrence of *P. yezoensis* at Dover Point, New Hampshire, USA, where it had been previously confused with a native species, *P. leucosticta* Thuret in Le Jolis. The *rbc*L and ITS1 sequences from the Dover Point material were sufficiently different from strains U51 and H25 for the author to rule out Coastal Plantations aquaculture site as the source for this introduction.

In the present paper the occurrence, distribution, molecular and morphological features are reported for New England populations of these two

Asiatic *Porphyra* species. In addition, the occurrences of three Texas populations of *Porphyra yezoensis* are documented along with morphological and molecular descriptions. Comparisons to original species descriptions are also provided.

Materials and Methods

Collections

The specimens examined in this study were from extensive recent field work as well as from historical collections of *Porphyra* from >900 sites within New England and the Canadian Maritime Provinces (cf. Coleman & Mathieson 1974; Mathieson 1979; Mathieson & Fralick 1972; Mathieson & Hehre 1986; Mathieson & Penniman 1986a,b, 1991; Mathieson et al. 1981, 1983, 1993, 1996, 1998, 2001, 2003), plus three locations within the Gulf Coast of Texas. The primary historical collections are located in the Albion R. Hodgdon Herbarium (NHA) at the University of New Hampshire. Specimens from other herbaria (BM, NY, FAR, PC, US, UPS, MICH and L) were also examined. Recent field collections were made at multiple sites, with specimens being collected at low tide from diverse intertidal and shallow subtidal habitats. Voucher specimens from these recent collections are deposited at NHA.

Molecular Methods

Tissue samples (~1 cm^2) were ground in a mortar and pestle, and genomic DNA was extracted using a Puregene Genomic DNA Purification kit. A

1467 bp fragment, from position 67 (amino acid 23) of the large subunit of *rbc*L through the *rbc*L-*rbc*S intergenic spacer to the first codon of the small subunit, was amplified with *rbc*L primers F67 and rbc-spc (Teasdale et al. 2002). The rbc-spc primer is selective for species of the Bangiales and does not amplify contaminating DNA from epiphytes that are common on macroalgal samples. The PCR reactions were carried out as detailed by Teasdale *et al.* (2002). The ITS1-5.8S-ITS2 region was amplified using the JBITS7 (Broom *et al.* 2002) and AB28 primers (Steane *et al.* 1991); ITS1 was sequenced using the JBITS7 and ITS1-R (5'-TATCCACCGTTAAGAGTTGTAT-3') primers. The PCR reagents and amplification profile were identical to those used by Teasdale *et al.* (2002). The resulting amplicons were gel-purified to confirm their size and to decrease the presence of non-specific or contaminating products prior to sequencing (Klein *et al.* 2003). The PCR amplified *rbc*L and ITS1 products were sequenced with an ABI 373 Automated Sequencer at the UNH Hubbard Center for Genome Sciences, using standard procedures outlined by Klein *et al.* (2003). Raw sequences were edited in Chromas (version 2.2, Technelysium, Pty. Ltd., Tewantin, Queensland, Australia). Contiguous sequence assembly and sequence alignments were done in SeqMan II and MegAlign (version 6.1 for Windows, DNAStar, Inc., Madison, Wisconsin, USA), respectively. Searches of GenBank were completed using BlastN via the Net Search option in MegAlign. The *rbc*L and ITS1 sequences for representative specimens were deposited in GenBank.

Phylogenies

Intraspecific genealogies were inferred in *Porphyra yezoensis* for ITS1 using the phylogenetic criteria of Maximum Parsimony (MP). Maximum Parsimony (MP) algorithims were preformed using the computer program PAUP* V.4.0 b10 (Swofford 2002) on a data matrix consisting of 12 taxa and 277 bp of the ITS1 region. Parsimony searches consisted of random sequence additions, MULTREES, tree bisection reconnection (TBR), and equal weights for all substitutions. Consistency (CI) and retention (RI) indices (Farris 1989, Kluge & Farris 1989) were calculated excluding uninformative characters. Support for nodes were determined by 1000 bootstrap replicates in order to asses the robustness of phylogenetic reconstruction.

Morphological and Ecological Assessments

Comparisons of several morphological features were made, including blade shape, color, margins, attachment, distribution of reproductive areas, and adherence to herbarium paper (cf. Neefus *et al.* 2002). Color measurements were recorded at several positions on each blade, using an X-Rite Digital Swatchbook Colorimeter; the values were averaged in Colorshop v.2.6.0 (X-Rite, Grandville, Michigan, USA). Color measurements are expressed in CIE L*a*b* tristimulus units (L* = lightness difference, a* = red/green difference, and b* = yellow/blue difference), which are based on a "standard observer" and are device-independent (Bunting 1998). Blade lengths, widths and shapes were also

recorded. Assessments of blade thickness, chloroplast morphology, number of cell layers, and division sequences of male gametangia and zygotosporangia were made from microscopic examination of surface and transverse sections of blades. The terminology for reproductive structures follows Nelson et al. (1999). All microscopy was done using an Olympus BX40 microscope, while microphotography was done with a Nikon D100 digital camera using Nikon Capture 3.0 software. Images were transferred to Adobe Photoshop 6.0.1 under Microsoft Windows XP on a Dell Precision 340 Workstation. Herbarium sheets used for figures were scanned with an Epson 1640XL flatbed scanner. Enumerations of seasonal, geographic and vertical distributions were made from herbarium label data, field notes and observation of *in situ* populations.

Results

Comparison of Asian and New England *Porphyra yezoensis*

Porphyra yezoensis Ueda 1932: p. 12 (key), 23, pl. I, Figure 9, 14, pl. IV, Figure 3.3.11-17, pl. XVI

Holotype: Miyagi Pref, northward, SW Hokkaido, N Honshu, Japan

Synonyms: *Porphyra palleola* Noda 1964: p. 9, Figure 8, *nom. inval.* See Guiry & Nic Dhonncha (2005) for synonymy.

Forms: f. *narawaensis* A. Miura 1984: p. 6, pl. 4, Figure 3.4, pl. 5, Figure 3.3.2, pl. 6, Figure 3.3-5, pl. 7, Figure 3.3.2-9, pls. 8-10

f. *yezoensis* Ueda 1932: p. 12 (key), 23, pl. I, Figure 9, 14, pl. IV, Figure 3.3.11-17, pl. XVI

Molecular Features. Molecular data confirmed the presence of two distinct genotypes of *Porphyra yezoensis* in New England and two genotypes in Texas (Tables 3.1-3.3; Figure 3.1, and Appendix B). In New England the two genotypes were f. *yezoensis* and f. *narawaensis.* The genotype f. *yezoensis* occurs from the western end of Long Island Sound to the mid-coast of Maine, while f. *narawaensis* occurs only south of Cape Cod (Figure 3.1). In Texas f. *yezoensis* was collected at three sites from Galveston to Port Isabel (Figure 3.2). The ITS1 sequence of the first genotype occurring in New England is identical to a wild specimen of *P. yezoensis* f. *yezoensis* collected near Nanaehama, Hakodate, Hokkaido, Japan, (NA-4, Kunimoto *et al.* 1999a, GenBank AB017076), while its *rbc*L sequence is 100% identical to a Japanese strain F6-1 (Kito, GenBank AB118590). Interestingly, the *rbc*L sequence of the Texas individuals were identical to *P. yezoensis* f. *yezoensis* occurring in New England; however, only the specimen from Port Isabel, Texas (GenBank DQ813582) collected in the late 1960's had an identical ITS1 sequence to the New England populations (Table 3.3). Maximum Parsimony analysis of the ITS1 sequence data showed contemporary Texas specimens collected from the other two Texas sites (Galveston GenBank DQ813580 and Port Aransas GenBank DQ813579) had a closer affinity to specimens (GenBank DQ649353, DQ649354) from China (Table 3.3, Figure 3.3).

The *rbc*L sequence of the second genotype found in New England was identical to several commercial Japanese cultivars of *P. yezoensis* f. *narawaensis*, including Saga-5 (Kito AB118587), Saga-5L (Kito AB118588),

Midorime (Kito AB118589), and U51 (Klein AF021032), plus isolates from Ogatsu Miyagi (Kito AB118575), and Hakodate Hokkaido (Kito AB118574). The ITS1 sequence from the second genotype found in New England was 100% identical to at least 16 commercial cultivars of f. *narawaensis* reported by Kunimoto *et al.* (1999a), including Saga-5 (GenBank AB019187), Saga-103 (GenBank AB017086), Noma-1 (GenBank AB017085), Midorime (GenBank AB017084), Fukuoka-1 (GenBank AB017082), G-1 (GenBank AB125321), HG-1 (GenBank AB125320), Harima-7 (GenBank AB125319), Noriken-15 (GenBank AB125318), Noriken-10 (GenBank AB125317), Noriken-4 (GenBank AB125316), 0110 (GenBank AB125315), Ariake-1 (GenBank AB019191), D-18-1 (GenBank AB019190), Obagreen (GenBank AB019189), and Sasiki (GenBank AB019188), plus two additional cultivars, H25 (Bray H-25 Connecticut GenBank DQ649356) and U51 (Bray U-51Connecticut, C. Yarish culture, GenBank AY569569).

Morphological Comparisons. The morphology of *Porphyra yezoensis* f. *yezoensis* populations from New England and Texas (Tables 3.1 & 3.2, Figure 3.4) is similar to the original description of *P. yezoensis* provided by Ueda (1932) and f. *yezoensis* given by Miura (1984). Blade shape is commonly ovate to oblong, but may become cuneate through erosion of the distal end during reproduction. Older specimens can become lacineate and irregular. Base shape may be round to cordate with a minute stipe. The tip is round to acuminate, but frequently eroded. The frond margins lack teeth, are generally unruffled in younger specimens, but may become moderately ruffled with age. Reproductive

blades range from 3-22 cm long by 1-21 cm wide, with length:width ratios varying from 1.5 to 4.5 (average 2.5). The color of freshly collected specimens is variable, ranging from brown to pinkish brown, mauve, and grey-green; color of dried specimens becomes distinctly pink to purple with time. Average L*a*b* color measurements for freshly pressed specimens are L=58.8, a=8.1, b=42.0, while average color values for specimens on older herbarium sheets are L=78.8, a=6.8, b=6.5. Blades are monostromatic. The vegetative portions of blades are 20-42 μm thick in New England populations and 27.1-39.5 μm thick in Texas populations, which agrees with Miura's (1984) measurement of Japanese f. *yezoensis* specimens (25-40 μm). According to Ueda (1932) Japanese specimens are thicker (32-53 μm). In surface view (SV) vegetative cells of New England and Texas plants are polygonal with rounded corners, and range from 7-24μm in length by 5-17 μm in width; in transverse section (TS) they are quadrate and 7.5-25 μm in height. Cells contain a single stellate plastid with a central pyrenoid. Blades are monoecious or androdioecious (i.e. with both monoecious and separate male fronds). Although Muira (1984) indicates that blades are occasionally trioecious, I could not confirm the occurrence of entirely female blades in either New England or Texas specimens. Male gametangia form pale marginal streaks, especially on distal portions of blades, while female gametangia develop in areas between male steaks. Male gametangial packets are arranged as 2(4) x 2(4) x 4 tiers of gametes [i.e. 2 (or 4) by 2(or 4) in SV by 4 tiers high in TS], while zygotosporangia occur as 2 x 2 x 2 tiers. Ueda (1932) describes 8 tiers of male gametes and 4 tiers of zygotospores, perhaps reflecting

thicker blades. Archeospores (single asexual reproductive cells) are common at the distal end of small blades, while endospores (irregular masses of asexual reproductive cells) are common near the base of blades.

Although the ITS sequence of the genotype that occurs in Long Island Sound is identical to cultivars of *Porphyra yezoensis* f. *narawaensis*, there are a number of morphological differences compared to Miura's (1984) original description. Foremost, the blade shapes of New England specimens range from linear to oblong to elliptical to suborbiculate, while Muira's (1984) description also includes a spirally twisted form that is frequently longer on one side than the other. I have not observed either of these conditions. The blade base of New England specimens is slightly to moderately cordate with a minute stipe, while Miura (1984) describes it as cuneate. The tips of New England specimens are acute to obtuse, which agrees with Miura (1984) description. Margins of New England specimens are entire, slightly ruffled and, in younger specimens, sometimes rolled. The tips and margins are frequently eroded and ragged due at least in part to the release of archeospores. Although the widths of specimens from both geographies are similar, the lengths of New England specimens (2-19 cm) are substantially shorter than those reported for Japan (40-100 cm). The color of freshly pressed New England specimens ranges from olive green to brown (average L=53.3, a=4.8, b=39.1), while older dried specimens become lighter and more pink or purple mauve (average L=78.4, a=6.2, b=7.5). By contrast, Muira (1984) describes the color of (presumably) dried specimens, as simply dark purplish.

The vegetative thalli of *P. yezoensis* f. *narawaensis* are monostromatic, with New England plants ranging in thickness from 25-47 μm (average 36μm) versus 25-30 μm in Miura's (1984) original description. In SV the cells of New England plants are quadrate or polygonal with have rounded corners, and range from 12-22 μm in length by 7.5-12.5 μm in width; in TS vegetative cells are rectangular, 12.5-15 μm tall, and have rounded corners. Muira (1984) describes vegetative cells as quadrate with rounded corners in SV, while they are subrectangular to elliptical in TS. Cells contain a single stellate chloroplast and a central pyrenoid.

Reproductive tissues differ from Miura's (1984) description, with male gametangia of New England specimens forming conspicuous, pale, tan marginal streaks, especially towards the distal end of the blade. Female gametangia develop in areas between male streaks and small blades may be reproductive. By contrast, Miura (1984) reports that male gametangia form minute spots and that reproductive maturation is retarded. In New England specimens, male gametangial packets are arranged as 2 x 2(4) x 4(8) tiers of gametes, and male portions of the blade are 35-57 μm thick. Miura (1984) reports thicker blades (40-70μm), with male gametangia arranged as 4 x 4 x 8(16) tiers. Female gametangial packets occur as 1(2) x 2 x 2 tiers in New England specimens versus 1(2) x 1(2) x 4 tiers in the type description.

Distribution and Ecology. In New England the blades of both genotypes of *Porphyra yezoensis* occur from January through April as does the one genotype (f. *yezoensis*) in Texas (Tables 3.1 & 3.2, Appendix B). Miura (1984) gives a

similar seasonality for f. *narawaensis*, with plants being "most luxuriant" from December through March (Table 3.2). As noted above, *P. yezoensis* f. *narawaensis* occurs only south of Cape Cod and has been collected from seven sites in Massachusetts, Rhode Island, and Connecticut (Figure 3.1, Appendix B). By contrast, f. *yezoensis* has a more extensive distribution in New England, having been collected at twenty sites from the mid-coastal Maine to the western end of Long Island Sound (Figure 3.1, Appendix B). In Texas f. *yezoensis* was collected at three sites along the inter-coastal waterway (Figure 3.2, Appendix B). Both genotypes grow primarily within tidal rivers, estuarine constrictions (tidal rapids), or coastal inlets, but they may also occur on semi-exposed to exposed open coastal sites. The two genotypes grow within the intertidal and shallow subtidal, with f. *yezoensis* extending to the upper intertidal and f. *narawaensis* being more common in the low intertidal and shallow subtidal, while either form can grow on rocks, shells, and other algae. Ueda (1932) states that *P. yezoensis* (f. *yezoensis*) grows intertidally on rocks and other algae, while Miura (1984) reports that f. *yezoensis* grows in the low intertidal, while f. *narawaensis* occurs in the very low intertidal; both forms grow on pebbles and shells.

Comparison of Asian and New England *Porphyra katadae*

Porphyra katadae A. Miura 1968: p. 55-58, pl. I-VII.

Holotype: Herbarium of the Tokyo University of Fisheries. March, 6. 1957. Ise Ominato, estuary of Miyagawa River, Mie Pref., Japan.

Molecular Features. The *rbc*L sequences (positions 77-1467) for *Porphyra katadae* specimens from Cape Cod Bay, Massachusetts were compared with cultures from Qingdao, China (PKSTF1 positions 1021-1467) provided by Tang and an existing GenBank sequence (AB118583, positions 1-1467 bp) submitted by Kito for a specimen from Kawatana, Yamaguchi (westernmost Honshu), Japan. The *rbc*L sequences were 100% identical across the overlapping regions. The ITS1 sequences (1-305 bp) for Cape Cod Bay specimens were also 100% identical to the Qingdao culture, but differed by 1 bp substitution from GenBank accession AB017090 submitted by Kunimoto *et al.* (1999a) for a specimen from Kawatana, Yamaguchi, Japan.

Morphological Comparisons. The morphology of *Porphyra katadae* from Cape Cod Bay, Massachusetts is nearly identical to Miura's (1968) original description from Japan (Table 3.4). Thus, there are two morphologies evident from both geographies: one is ruffled and elongate (Figure 3.5A-B), while the other is unruffled and ovate (Figure 3.5C-E). The two morphologies are reflected in the dimensions and shapes detailed in Table 3.4. In both forms, their bases are cordate to pseudoumbilicate and coloration is a pale, purplish red. Specimens from New England average 31 µm (27-39 µm) in thallus thickness compared to 20-30 µm in Japan. Reproductive individuals are longitudinally sectored into reddish female and pale yellow male "halves". Sectoring is most conspicuous in the ovate form. Male portions of the thallus appear to mature first and then erode away leaving a falcate or "comma-shaped" blade. Miura (1968) describes the species as androdioecious, while we have only observed several

elongate non-sectored male plants. While Miura (1968) records male gametangial packet arrangement of 4 tiers of 4x4 cells, I have found 4 tiers of 4x2 to be more common. By contrast, the Chinese variety *Porphyra katadae* var. *hemiphylla* has a thicker thallus (35-45 μm) and its male gametangial packets are 8 tiers high.

Distribution and Ecology. *Porphyra katadae* has been collected at three locations in Cape Cod Bay, Massachusetts (Eastham, Barnstable, and Sandwich), plus one site in Buzzard's Bay near Bourne (Figure 3.1). The first two sites are sandy, shallow embayments, while the third and fourth are rocky, fast current areas at the north and south ends of the Cape Cod Canal. The species occurs within the low intertidal and shallow subtidal during March and April. In sandy locations, *P. katadae* is elongate and ruffled, and grows epiphytically on *Gracilaria tikvahiae* McLachlan, which in turn is attached to pebbles. Specimens from the Canal sites are round and unruffled, and they grow attached to *Chondrus crispus* Stackhouse and *Dumontia contorta* (S.G.Gmelin) Ruprecht.

Discussion

The present study provides strong evidence that populations of *Porphyra yezoensis* in New England and Texas have been introduced from Asia. The occurrence of two distinct genotypes in New England provides evidence of at least two introductions, with one (i.e. *Porphyra yezoensis* f. *narawensis*) having a more circumscribed distribution in eastern Long Island Sound (Figure 3.1) and

matching a commercial cultivar that has been widely grown in Japan since the 1980's. Such a pattern suggests a recent single introduction of this genotype. However, it should be noted that all commercial Japanese cultivars of f. *narawensis* have identical *rbc*L and ITS1 sequences and the form could have been introduced more than once. Based upon field studies the only way one could determine if multiple introductions had occurred would be if disjunct (segmented) populations were evident; however, the distribution of f. *narawensis* is continuous and restricted to Long Island Sound. Thus, it is likely it represents a single introduction that occurred after 1980.

Porphyra yezoensis f. *yezoensis* occurs in New England and the Gulf Coast of Texas (Figures 3.1 & 3.2). In New England, f. *yezoensis* has a wider distribution than f. *narawaensis* and its ITS1 sequence matches a wild non-cultivated specimen from Japan. The plant occurs from mid-coastal Maine to the western end of Long Island Sound and perhaps farther south (Figure 3.1). Its distribution is interrupted within the eastern end of Long Island Sound where f. *narawensis* occurs (Figure 3.1). To date I have found no sites where both genotypes occur together. Herbarium specimens of *P. yezoensis* f. *yezoensis* date back to the mid-1960's at Dover Point NH where Reynolds (1971) identified it as *P. leucosticta*. By contrast a recent comprehensive study at the same site by West et al. (2005) found no *P. leucosticta*, while *P. yezoensis* f. *yezoensis* was common from January– May. Thus, it is likely that the species has been at Dover Point, NH for 40 or more years and not distinguished from native species. In Texas, the occurrence of *P. yezoensis* f. *yezoensis* having 100% identical *rbc*L

and ITS1 sequences to contemporary New England populations has been molecularly confirmed from a specimen collected in 1969 from Port Isabel (GenBank DQ813582), while recent specimens from Galveston (GenBank DQ813580) and Port Aransas, Texas (GenBank DQ813579) have ITS1 sequences with a closer affinity to Chinese specimens (GenBank DQ649353, DQ649354). The occurrence of f. *yezoensis* in Texas with two different ITS1 sequences would seem to indicate multiple introductions, but from different points of origin.

Wild specimens of f. *yezoensis* from Japan exhibit variation of ITS1 sequences among individuals within a single population. However, we found no such variation among different individuals or sites in New England. Thus, I suspect that the distribution of f. *yezoensis* was at one time continuous between mid-coastal Maine and the western end of Long Island Sound; however, its distribution is now fragmented in eastern Long Island Sound as a result of the more recent introduction of an aggressive cultivar of f. *narawensis*. Miura & Aruga (1987) report that extensive populations of f. *narawaensis* have become established on natural substrata in Japanese culture areas, resulting in the displacement of other *Porphyra* species. They also state that *P. yezoensis* f. *narawensis* has altered the community structure and ecology of the Japanese coastal areas, resulting in decreased genetic diversity. I do not know what effect either genotype has had on native species or community structure. Both genotypes, but especially f. *narawensis,* occur as very dense epiphytic

populations on several native perennial seaweeds, including *Fucus vesiculosus*, *Chondrus crispus* Stackhouse, and *Mastocarpus stellatus* (Stackhouse) Batters.

Unlike New England populations, variation in the ITS1 sequence has been found between geographically and temporally distinct Texas populations of *P. yezoensis* f. *yezoensis* suggesting multiple introductions of this taxa. To date, no populations of f. *narawensis* have been found along the Texas gulf coast.

In discussing the occurrence of *Porphyra yezoensis* at Dover Point, New Hampshire, West et al. (2005) ruled out the Eastport, Maine nori aquaculture operation (cf. Levine 1998) as a source of this introduction, because it *rbc*L sequence differed by 2 bp from the U51 cultivar grown at the site (Klein et al. 2003). My results confirm this conclusion, since the f. *narawensis* genotype was grown in Eastport, while f. *yezoensis* occurs at Dover Point, New Hampshire. Based upon sequence data alone we cannot rule out the Eastport operation as a source of the f. *narawensis* introduction to Long Island Sound. However, I do not believe that this is the case for several reasons. Foremost, virtually all commercial strains of f. *narawensis* have the same *rbc*L and ITS1 sequences, so the source of the Long Island Sound population could have been any one of many Japanese nori farms. Secondly, the lack of f. *narawensis* populations between Eastport and Cape Cod argues against its spread by the prevailing south flowing and counter-clockwise currents within the Gulf of Maine, with these water masses being isolated from Long Island Sound (Apollonio 1979). With respect to boat transfer (i.e. by fouling or ballast water), there is undoubtedly more shipping from Japan to Long Island Sound than from Eastport to Long

Island Sound. Some investigators have suggested that other Asiatic seaweeds (e.g. *Codium fragile* ssp. *tomentosoides*) were introduced to Long Island via shellfish aquaculture (Malinowski, 1974), while others (Carlton and Scanlon 1985) emphasize that ships (i.e. their hulls) were more likely vectors.

Similar to New England *Porphyra yezoensis* f. *yezoensis* populations, contemporary specimens from Galveston and Port Aransas, Texas, USA have no variation in ITS1 sequences. However, the ITS1 sequences from the recent Texas populations did not match Japanese specimens, but rather have a closer affinity to Chinese individuals (Table 3.3 & Figure 3.3). This difference in ITS1 sequences indicates that introduced *P. yezoensis* f. *yezoensis* populations in New England and Texas had different sources. Initially identified as *P. leucosticta,* an herbarium sample from Port Aransas dating back to the late 1960's has been molecularly identified as *P. yezoensis* f. *yezoensis* (Appendix B). However, given the limited number of collections examined and the numerous opportunities for coastal spread (i.e. ship traffic) within the inter-coastal waterway, it is not possible to determine an introduction site and complete distributional pattern for *P. yezoensis* f. *yezoensis* in the Gulf of Mexico.

At this point I cannot establish a source for the introduction of the New England population of *Porphyra katadae* as its *rbc*L sequence matches (100%) both Japanese and Chinese specimens. The one bp substitution in its ITS1 sequence vs. Japanese specimens is not significant without knowing how much variation exists within other Asiatic populations. My initial studies indicate *P. katadae* has a very circumscribed distribution near the Cape Cod region and its

local distribution appears to be associated with the Cape Cod Canal and possibly boat traffic. Even so, the species may be more broadly distributed than currently recognized, as it has been previously confused with several other taxa— i.e. *P. purpurea* (Roth) C. Agardh (cf. Mitman 1991; Bird and McLachlan 1992), and *P. umbilicalis* Kützing (cf. Taylor, 1966). The presence of *P. katadae* on the northeast coast of North American may also help to explain variable reports of chromosome counts for *P. purpurea* (Wilkes et al. 1999).

In summary, although there have been few documented cases of *Porphyra* introductions, I suspect that the occurrence is more common than previously recognized. Such introductions may have important implications for biodiversity assessments/comparisons, as well as taxonomic and systematic evaluations of the genus. Further, introductions may have considerable ecological impacts. The evaluation of "sister taxa" within different ocean basins (e.g. *P. birdiae* vs. *P. aestivalis*) is an important initial step to making such assessments, as some recently described species (e.g. *P. carolinensis* and *P. lilliputiana*) have been shown to be introduced, synonymous taxa (Broom et al. 2002).

Table 3.1. A comparison of New England *Porphyra yezoensis* f. *yezoensis* with Ueda and Miura descriptions from Japan.

	Porphyra yezoensis f. *yezoensis*		
	New England and Texas This study	**Japan Ueda (1932)**	**Japan Miura (1984)**
Morphology			
Shape	Ovate to oblong, becoming cuneate due to erosion of distal end. Occasionally elongate. Older specimens can be irregular. Some individuals split longitudinally giving the appearance of multiple blades.	Ovate, oblong-ovate, round or lanceolate.	Obdeltoid to round through broadly oblaceolate, elliptical obdeltoid, suborbiculate to falcate.
Base	Round to cordate with a minute stipe.	Round to cordate with extremely short stipe.	
Tip	Round to acuminate, but frequently eroded		
Margins	Entire, young specimens mostly unruffled, older specimens moderately ruffled.	Entire (no teeth). Scattered deep folds.	
Dimensions	Length 3-22 cm. Width 1-21 cm. Length:width ratio1.5-4.9 (average 2.5)	Length 10-20 cm. Width 3-13 cm.	Length 10-15 cm.
Color	Brown, pinkish brown, mauve or gray-green (average L*a*b* 58.8, 8.1, 42.0), becoming more distinctly pink to purple over time after drying. (average L*a*b* 78.8, 6.8, 6.5).	Bright purple (violet).	
Thallus Thickness	Monostromatic 20-42.0 µm (average 32.0 µm) in vegetative areas, 25-35 µm (average 32.0 µm) in reproductive areas.	Monostromatic 32-53 µm.	Monostromatic 25-40 µm.
Vegetative Cells	5.0-17.0 µm x 7.0-24.0 µm in SV, 7.5-25.0 µm tall in TS.	Polygonal or slightly angular round in SV. Quadrate with rounded corners in TS, twice as tall as broad.	
Chloroplasts	Single, stellate with central pyrenoid.		

Table 3.1. Continued.

	Porphyra yezoensis f. *yezoensis*		
	New England and Texas This study	**Japan Ueda (1932)**	**Japan Miura (1984)**
Reproduction			
Distribution of Reproductive Tissues	Monoecious or androdioecious. Male gametangia forming macroscopic pale marginal streaks, especially on the distal portion of the blade. Female gametangia develop in areas between the male streaks.	Monoecious. Male and female forming patches first on the margin and then mixed with each other to form a slightly striped, splashed pattern. In some small sections, male and female cells arranged with slight irregularity.	Mostly monoecious, occasionally andro-dioecious or trioecious; formed in the whole upper part of the frond as marginal streaks .
Male Gametangial Packet Arrangement	2(4) x 2(4) x 4 tiers.	4 x 2 x 8 tiers.	
Zygotosporangial Packet Arrangement	2 x 2 x 2 tiers.	2 x 2 x 4 tiers.	
Archeosporangia (monosporangia)	Common in small specimens, causing erosion of the distal end of the blade		Common.
Endosporangia	Common near base of blade.		
Ecology			
Seasonality	January to May.		
Habitat	Tidal rivers, estuarine constrictions and coast inlets with strong tidal currents and rocky substrate. Also on semi-exposed to exposed open coastal areas with rocky substrate.	Every coast.	
Elevation	High intertidal to shallow subtidal, more common in lower intertidal.	Intertidal.	Low intertidal.
Substrata	Occasionally epilithic, but mostly epiphytic on other algae including *Dumontia contorta, Fucus vesiculosus, Syitosiphon lomentaria,* and *Mastocarpus stellatus.*	Rocks and other algae.	Pebbles and shells.

Table 3.2. A comparison of New England *Porphyra yezoensis* f. *narawaensis* with Miura descriptions from Japan.

	Porphyra yezoensis f. *narawaensis*	
	New England **This study**	**Japan** **Miura (1984)**
Morphology		
Shape	Linear, lanceolate, oblong, elliptical, to suborbiculate; frequently irregular.	Linear, oblanceolate or ovate, occasionally laciniate or spirally twisted; frequently one side is longer than the other.
Base	Slightly to moderately cordate with a minute stipe.	Cuneate.
Tip	Acute to obtuse; frequently eroded and ragged.	Acuminate, acutate or obtuse.
Margins	Entire, slightly ruffled; sometimes rolled (in small specimens); frequently eroded and irregular.	Entire (no teeth), slightly folded.
Dimensions	Length 2-19 (average 9.3) cm. Width 1-7 (average 3.2) cm. Length:width ratio 1.0-12 (average 3.4).	Length 40-100 cm. Width to 3-7 cm.
Color	Fresh specimens olive green to brown (average L*a*b* 53.3, 4.8, 39.1) becoming lighter and more pink to purple mauve (average L*a*b* 78.4, 6.2, 7.5) with time after drying.	Dark purplish.
Thallus Thickness	Monostromatic 25-47 (average 36) µm.	Monostromatic 25-30 µm.
Vegetative Cells	Quadrate to polygonal with rounded corners 7.5-12.5 µm x 12-22 µm in SV; rectangular with rounded corners 9.8-22.2 µm tall in TS.	Quadrate with round corners in SV; subrectangular or elliptical in TS.
Chloroplasts	Single, stellate with central pyrenoid.	Single, stellate.

Table 3.2. Continued.

	Porphyra yezoensis f. *narawaensis*	
	New England	**Japan**
Reproduction	**This study**	**Miura (1984)**
Distribution of Reproductive Tissues	Monoecious. Male gametangia forming pale tan streaks marginal streaks especially in the distal half of the blade. Female gametangia form in areas between the male streaks. Even small blades can be reproductive.	Always monoecious, maturation retarded, male gametangia forming minute spots or small patches amoung zygotosporangia; male gametes released before zygotospores; male gametangia and zygotosporangia form along distal margin in a splashed pattern.
Male Gametangial Packet Arrangement	2 x 2(4) x 4(8) tiers; Blade 37-57 µm thick.	4 x 4 x 8 (16) tiers; Blade 40-70 µm thick.
Zygotosporangial Packet Arrangement	1(2) x 2 x 2 tiers; Blade 37.5-45 µm thick.	1(or 2) x 1(or 2) x 4 tiers;carpogonia with conspicuous trichogynes. Blade 40 µm thick;
Archeosporangia (monosporangia)		Present in fronds greater than 0.5 to 4.0 cm, but less active than in f. *yezoensis.*
Ecology		
Seasonality	January to April.	Most luxuriant from December to March.
Habitat	Sheltered to exposed coastal.	Estuarine, coastal.
Elevation	Low to shallow subtidal; occasionally mid to high intertidal.	Very low intertidal.
Substrata	Common on *Chondrus crispus* , *Dumontia contorta* ; occasionally on *Fucus vesiculosus* or rock.	Pebbles and shells.

Table 3.3. Percent divergence between ITS1 sequences (277 bp) from wild and cultured strains of *Porphyra yezoensis* f. *yezoensis* and *P. yezoensis* f. *narawaensis* collected from Japan, China, New England, and Texas. *P. y.* f. *y.* = *P. yezoensis* f. *yezoensis, P. y.* f. *n.* = *P. yezoensis* f. *narawaensis.*

	P. y. f. *y.* (Port Aransas, TX, USA)	*P. y.* f. *y.* (Galveston, TX, USA)	*P. y.* f. *y.* (China)	*P. y.* f. *y.* (Qingdoa, China)	*P. y.* f. *y.* (Port Isabel, TX, USA)	*P. y.* f. *y.* (Massachusetts, USA)	*P. y.* f. *y.* (New York, USA)	*P. y.* f. *y.* NA-4 (Japan)	*P. y.* f. *n.* Midorime (Japan)	*P. y.* f. *n.* (Massachusetts, USA)	*P. y.* f. *n.* Saga-5 (Japan)	*P. y.* f. *n.* Noma-1 (Japan)
P. y. f. *y.* (Port Aransas, TX, USA)												
P. y. f. *y.* (Galveston, TX, USA)	0.0											
P. y. f. *y.* (China)	0.6	0.6										
P. y. f. *y.* (Qingdoa, China)	0.3	0.3	0.3									
P. y. f. *y.* (Port Isabel, TX, USA)	2.3	2.3	1.6	1.9								
P. y. f. *y.* (Massachusetts, USA)	2.3	2.3	1.6	1.9	0.0							
P. y. f. *y.* (New York, USA)	2.3	2.3	1.6	1.9	0.0	0.0						
P. y. f. *y.* NA-4 (Japan)	2.3	2.3	1.6	1.9	0.0	0.0	0.0					
P. y. f. *n.* Midorime (Japan)	2.9	2.9	2.2	2.6	0.6	0.6	0.6	0.6				
P. y. f. *n.* (Massachusetts, USA)	2.9	2.9	2.2	2.6	0.6	0.6	0.6	0.6	0.0			
P. y. f. *n.* Saga-5 (Japan)	2.9	2.9	2.2	2.6	0.6	0.6	0.6	0.6	0.0	0.0		
P. y. f. *n.* Noma-1 (Japan)	2.9	2.9	2.2	2.6	0.6	0.6	0.6	0.6	0.0	0.0	0.0	

Table 3.4. A comparison of New England *Porphyra katadae* with Muira's original description from Japan.

	New England This study	Japan Miura (1968)
Morphology		
Shape	Round, ovate, lanceolate, elongate, falcate.	Ovate, lanceolate, falcate ("comma-like").
Base	Cordate, pseudo-umbilicate	Cordate, umbilicate
Margins	Entire, ruffled in lanceolate specimens	Entire (no teeth), deeply folded.
Dimensions	Length 8-20cm (average 15cm). Width 3-16cm (average 12cm).	Length 5-15cm (up to 30). Width 2-6cm (up to 16).
Color	Fresh specimens reddish brown (average L*a*b* 62.03, 10.91, 34.83), becoming pale purplish red (average L*a*b* 80.9, 7.2, 6.2) over time.	Pale purplish red.
Thallus Thickness	27-39 µm. (average 31 µm)	20-30 µm.
Vegetative Cells	7.4-17.2 µm x 7.4-22.2 µm in SV, 9.8-27.5 µm tall in TS.	
Chloroplasts	Single, stellate with central pyrenoid.	
Adherence to Paper	Adheres well	
Reproduction		
Distribution of Reproductive Tissue	Monoecious, longitudinally sectored into reddish female and pale yellow male halves.	Androdioecious with homothallic individuals separated by a median longitudinal line into reddish female and yellowish male halves.
Male Gametangial Packet Arrangement	4x2(4)x4 tiers.	4x4x4 tiers.
Zygotosporangial Packet Arrangement	2x2x2 tiers.	
Ecology		
Seasonality	Spring (March, April).	Mostly February-March (additional records from January, June, October).
Habitat	Shallow embayments, tidal rapids.	Estuarine, coastal.
Elevation	Low intertidal, shallow subtidal.	Intertidal, shallow subtidal, or tide pools.
Substrata	Epiphytic on *Gracilaria tikvahiae, Chondrus crispus or Dumontia contorta*	Epiphytic on *Grateloupia filicina* v. *porracea* or epilithic.

Figure 3.1. Distributional patterns of *Porphyra yezoensis* f. *yezoensis*, *P. yezoensis* f. *narawaensis*, and *P. katadae* in New England.

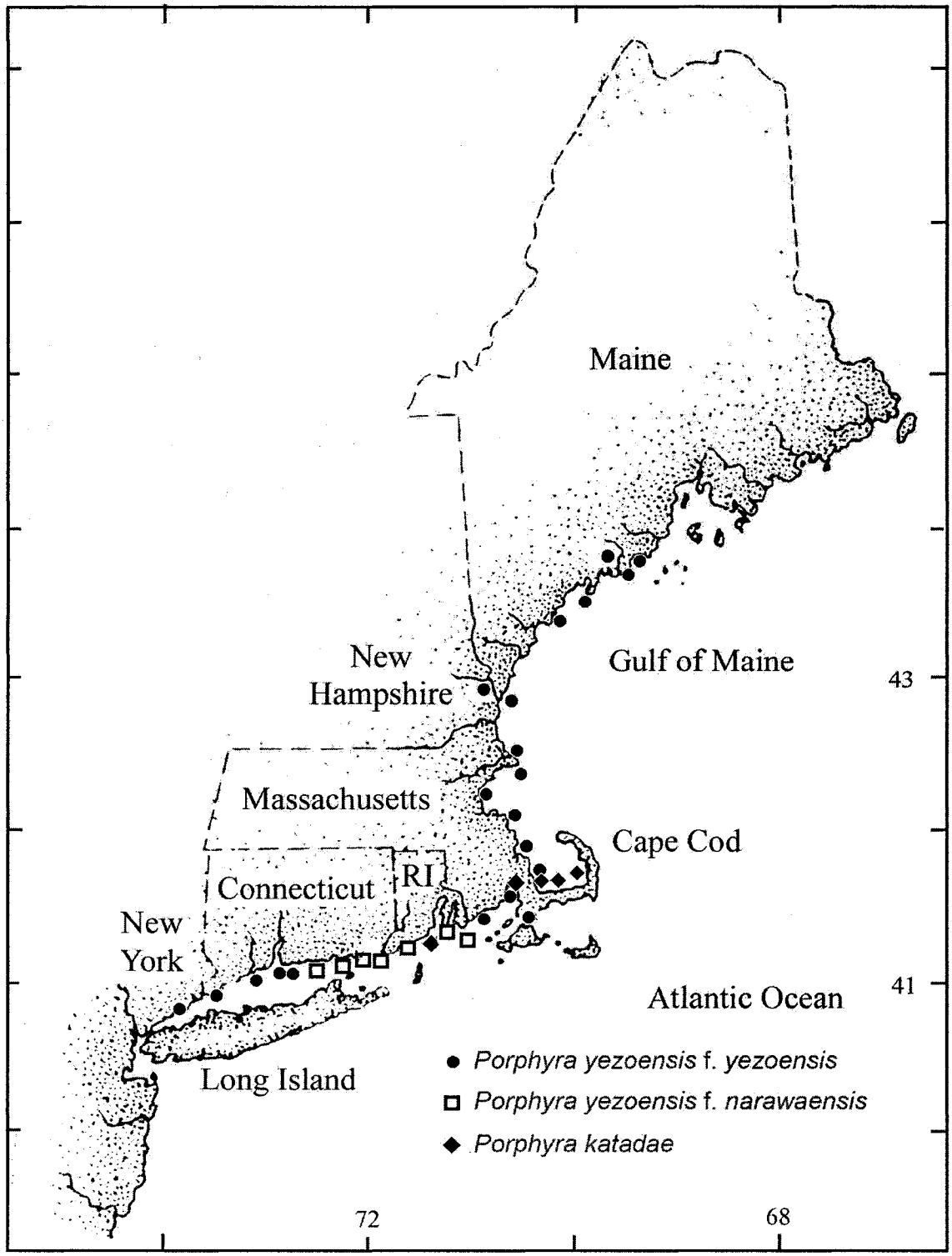


Figure 3.2. Three collection sites of *Porphyra yezoensis* f. *yezoensis* on the Gulf Coast of Texas.

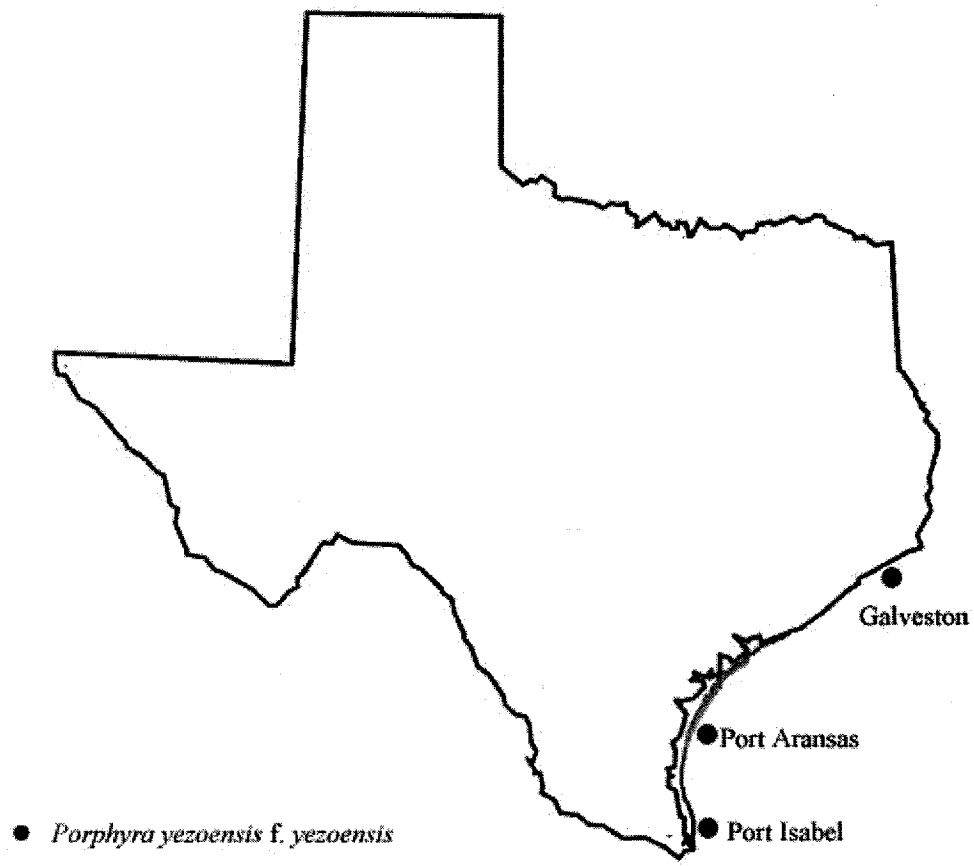


Figure 3.3. Single most parsimonious tree based upon 277 bp of the ITS1 region from *Porphyra yezoensis* using the evolutionary criterion of Maximum Parsimony. Tree length = 32 steps, CI=0.938, RI=0.923, RC=0.865. Numbers represent bootstrap support values (1000 replicates). *P. y.* f. *y* = *P. yezoensis* f. *yezoensis*, *P. y.* f. *n* = *P. yezoensis* f. *narawaensis*.

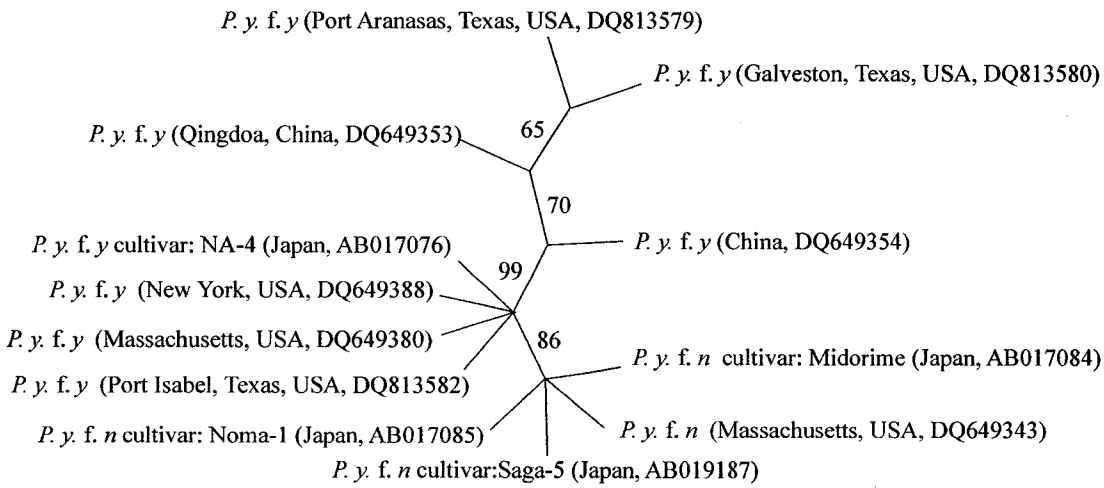


Figure 3.4. Various blade morphologies of *Porphyra yezoensis* f. *yezoensis* (A – H) in New England and Texas and *P. yezoensis* f. *narawaensis* (I – L) in New England.

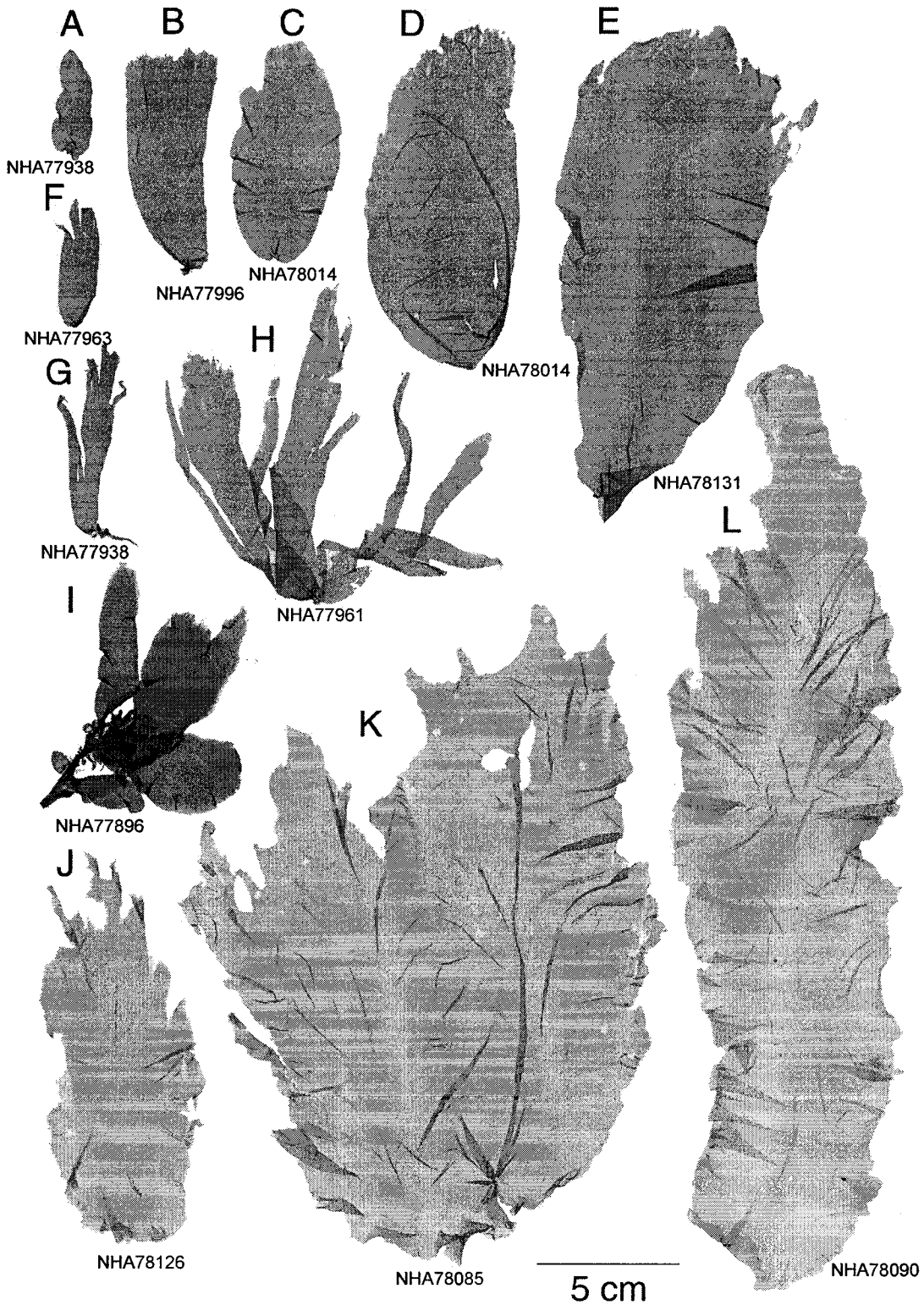
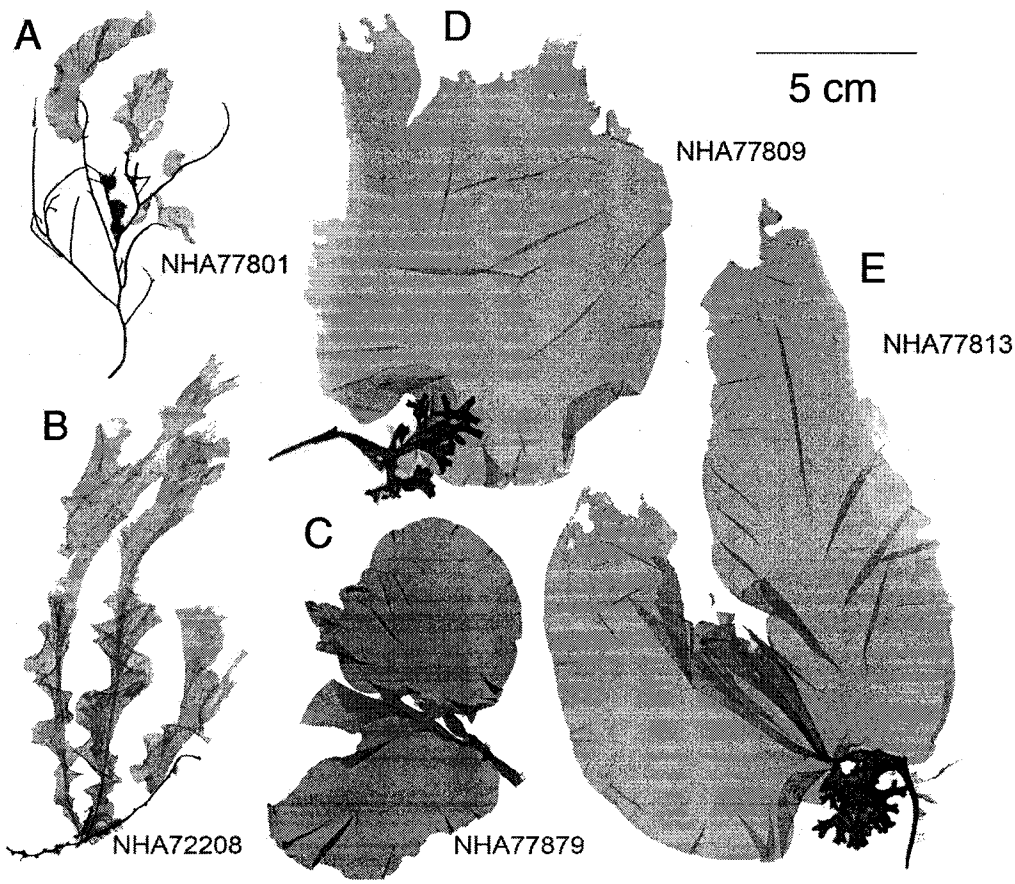


Figure 3.5. Two forms of *Porphyra katadae* blade morphologies in the Northwest Atlantic: ruffled and elongate (A – B) and unruffled and ovate (C – E).



CHAPTER IV

THE OCCURRENCE OF FIVE CRYPTIC SPECIES OF *PORPHYRA* IN THE NORTHWEST ATLANTIC

Abstract

Due to the paucity of distinguishing morphological and ecological characters, an underestimation of species diversity in the red algal genus *Porphyra* C. Agardh has been suspected as some species limits have been too broadly interpreted. However, with the advent of molecular techniques and the addition of sequence data as another taxonomic character, local floras continue to reveal unexpected diversity. Historically, only six species of *Porphyra* were reported from the Northwest Atlantic. Using sequence data from the chloroplast gene, *ribulose bisphosphate carboxylase oxygenase large subunit* (*rbc*L) and the nuclear ribosomal DNA internal transcribed spacer region (ITS1) as well as segments of the ribosomal RNA Small Subunit (SSU) five cryptic species of *Porphyra* are reported along the New England coast. Four of the five species are herein described as new species of *Porphya*. The occurrence, distribution, molecular and morphological features are of all five species are reported.

Introduction

The red algal genus *Porphyra* C. Agardh is ubiquitous within rocky intertidal habitats of temperate coastal areas. A few select species have been cultivated for centuries and processed into the edible seafood "nori," which continues to be the basis for one of the world's largest aquaculture industries (Mumford & Miura 1988). Despite its economic importance, the taxonomy of *Porphyra* and the ability to identify species continues to be problematic due to the paucity of distinguishing morphological and ecological features (Brodie et al. 1998, Klein et al. 2003, West et al. 2005, Bray et al. 2006). As a result, species diversity has been underestimated worldwide (Brodie & Irvine 1997, Neefus et al. 2002, Lindstrom & Fredericq 2003, Farr et al. 2003).

In the Northwest Atlantic, Bird and McLachlan (1992) suspected that the limits of some species of *Porphyra* were too broadly interpreted and that some taxa were in fact "form species" comprised of a number of similar but cryptically distinct entities. The suspicion was proven valid with the recognition of two species confirmed by molecular data, *Porphyra birdiae* Neefus & Mathieson, a newly described species (Neefus et al. 2002) and the reported occurrence of the Asiatic *Porphyra katadae* Miura (Neefus et al. In press), both of which have male and female reproductive tissue separated on distinct "halves" or "sectors" on their blades. Previously, all monostromatic *Porphyras* with sectored blades in the Northwest Atlantic were misidentified as *Porphyra purpurea* (Roth) C. Agardh

(Bird & McLachlan 1992, Taylor 1957). Another species suspected of being a "form species" in the Northwest Atlantic is *Porphyra leucosticta* Thuret *in* Le Jolis. *Porphyra* specimens from the North Atlantic with male reproductive tissue arranged in marginal "streaks" or "patches" have been historically identified as *P. leucosticta* (Bird & McLachlan 1992, Taylor 1957); however, recent reports of the Asiatic species *Porphyra yezoensis* Ueda in New England (West et al. 2004) have shown that this form species concept is incorrect. The occurrence of such "form species" concepts has become even more problematic in local floras with the introduction of nonendemic species having similar morphologies.

Molecular tools have been critical in revealing cryptic diversity within the genus *Porphyra* in the northwest Atlantic (Neefus et al. 2002). Historically, only six species of *Porphyra* were recorded from New England and the Canadian Maritime Provinces: *P. amplissima* (Kjellman) Setchell & Hus *in* Hus, *P. leucosticta*, *P. linearis* Greville, *P. miniata* (C. Agardh) C. Agardh, *P. purpurea*, and *P. umbilicalis* Kützing (Taylor 1957, Mathieson & Hehre 1986, Bird & McLachlan 1992, Silva 1999). Based upon a combination of DNA sequencing and detailed morphological and ecological studies, the list of species occurring in this geographical region continues to grow with additions of new described species such as *P. birdiae* (Neefus et al. 2002), as well as new reports of introduced species such as *P. yezoensis, P. katadae, and P. suborbiculata* Kjellman (Neefus et al. in press).

Sequences of the large subunit of the ribulose bisphophate carboxylase/oxygenase gene (*rbc*L) and the small subunit ribosomal DNA (SSU)

have been shown to distinguish morphologically similar *Porphyra* species (Neefus et al. 2002, Klein et al. 2003, Lindstrom & Fredericq 2003, Stiller & Waaland 1993, West et al. 2005). In addition, sequence data from the non-coding, nuclear ribosomal DNA internal transcribed spacer (ITS) has been used for intraspecific delineation and phylogenetic analyses (Kunimoto 1999a,b, Niwa et al. 2005).

In my investigations of *Porphyra* taxa from the northeast coast of North America, molecular data was used to help identify specimens from extensive collections of *Porphyra* from Long Island Sound to Cobscook Bay, Maine. Sequence data (*rbc*L, SSU, ITS1), in conjunction with ecological, anatomical, and morphological characters, were used to delineate five cryptic species found along the New England coast. Herewith, I give detailed descriptions of these five taxa including documentations of their temporal and spatial patterns.

Materials and Methods

Collections

The specimens examined in this study were from extensive recent and historical collections of *Porphyra* representing >900 sites within New England and the Canadian Maritime Provinces. The primary historical collections are located in the Albion R. Hodgdon Herbarium (NHA) at the University of New Hampshire. Specimens from other herbaria (BM, NY, FAR, PC, US, UPS, MICH and L) were also examined. Additional field collections were made at multiple sites during winter and spring of 2003 to 2005, with specimens being collected at

low tide from diverse intertidal and shallow subtidal habitats. Voucher specimens from these recent collections are deposited at NHA (Appendix C).

Molecular Methods

Extractions and amplification methods for *rbc*L and ITS1 region were identical to those used in Chapter 2, while the SSU was amplified using primers (Por 3 and Por 4) and protocols identical to Klein et al. (2003).

Sequence Alignments and Phylogenetic Analyses

Alignments for all sequences were done using the Clustal V or W methods in MegAlign (version 6 for Windows, DNA Star, Inc., Madison, Wisconsin, USA). Searches of GenBank were completed using BlastN via the Net Search option in MegAlign. Partial *rbc*L, SSU, *rbc*L-*rbc*S spacer and ITS1 sequences for representative specimens of each species were submitted to GenBank. Phylogenetic analyses of trimmed *rbc*L sequences (1077 bp from position 390 of the *rbc*L to position 27 of the *rbc*L-*rbc*S spacer) from this study plus GenBank sequences for other *Porphyra* species from the North Atlantic were carried out via Bayesian, Neighbor Joining (NJ) and Maximum Parsimony (MP) methods.

Bayesian Analysis was completed with MrBayes (version 3.1) using the General Time Reversible evolutionary model with the rate model varying according to codon position. It was run for 10,000,000 generations with a sampling frequency of 100 and burn-in set to 100,000 trees. The tree was converted to Windows Meta-file (WMF) format using TreeView for Windows

(version 1.6.6), and was prepared for final publication using Abobe Illustrator (version 9.0).

Maxiumum Parsimony and Neighbor Joining trees were constructed using the computer program PAUP* V.4.0b10 (Swofford 1998). Parsimony searches consisted of random sequence additions, MULTREES, tree bisection reconnection (TBR), and equal weights for all substitutions. Consistency (CI) and retention (RI) indices (Farris 1989, Kluge & Farris 1989) were calculated excluding uninformative characters. The neighbor-joining (NJ) was used to construct phylogenetic hypotheses for *rbc*L. Uncorrected distances with among-site rate variation was used with an NJ algorithm to construct the *rbc*L phylogeny under the criterion of minimum evolution (ME).

The resulting trees were rooted using a GenBank sequences (AF087119) for *Smithora naiadum* (C. L. Anderson) G. J. Hollenberg as an outgroup. Support for nodes of the MP and NJ tree were determined by calculating bootstrap proportion values based on 1000 resamplings.

Morphological and Ecological Assessments

Comparisons of morphological features were made, including blade shape, color, margins, attachment, distribution of reproductive areas, and adherence to herbarium paper (cf. Neefus *et al.* 2002). Color measurements were recorded at several positions on each blade, using an X-Rite Digital Swatchbook Colorimeter; the values were averaged in Colorshop v.2.6.0 (X-Rite, Grandville, Michigan, USA). Color measurements are expressed in CIE L*a*b* tristimulus units, which

are based on a "standard observer" and are device-independent (Bunting 1998). Blade lengths, widths and shapes were also recorded. Assessments of blade thickness, chloroplast morphology, number of cell layers, and division sequences of male gametangia and zygotosporangia were made from microscopic examination of surface and transverse sections of blades. The terminology for reproductive structures follows Nelson *et al.* (1999). All microscopy was done using an Olympus BX40 microscope, while microphotography was done with a Nikon D100 digital camera using Nikon Capture 3.0 software. Images were transferred to Adobe Photoshop 6.0.1 under Microsoft Windows XP on a Dell Precision 340 Workstation. Herbarium sheets used for figures were scanned with an Epson 1640XL flatbed scanner. Enumerations of seasonal, geographic and vertical distributions were made from herbarium label data, field notes and observation of *in situ* populations.

Results

To confirm the legitimacy of new taxa, *rbc*L, ITS1, and SSU sequences for each taxa were compared with all *Porphyra* assessions available on GenBank, including ~ 60 described and ~ 40 undescribed taxa. The sequence data for all taxa were unique and different from all those recorded in GenBank. Morphological and ecological characters for the four new taxa were compared and found different from the original descriptions of 15 *Porphyras* having reproductive streaks or patches for which no sequence data is reported on GenBank; these include *P. ishigecola* A. Miura, *P. moriensis* H. Ohmi, *P. pujalsii*

J. Coll *et* E. C. Oliveira, *P. rizzinii* J. Coll *et* E.C. Oliveira Filho, *P. maculosa* E. Conway, *P. aeodis* Griffin, Bolton *et* Anderson, *P. chauhanii* C. A. Kumar *et* M. V. N. Panikkar, *P. yamadae* Yoshida, *P. crispate* Kjellam, *P. ishigecola* Miura, *P. gardneri* (Smith *et* Hollenberg) Hawkes, *P. indica* V. Krishnamurthy *et* M. Baluswami, *P. thuretii* Setchell *et* Dawson, *P. cameronii* W. A. Nelson, and *P. tasa* (Yendo) Ueda.

However, upon further comparisons of sequence data to an undescribed Mediterranean taxon, for which no GenBank submission had been made, one "New England taxon" was found to have an identical partial SSU sequence as well as a similar morphology. Working with European collaborators, this taxon is currently being described as a new species, *Porphyra olivii* Orfanidis, Neefus, & Bray (Brodie et al., submitted). Data based on Northwest Atlantic populations is presented below.

Porphyra olivii Orfanidis, Neefus, & Bray

Holotype. BM000806051. Collected from North Krini, Thessaloniki Gulf, Greece.

Isotype. No specimens.

Etymology. The name *Porphyra olivii* commemorates G. Olivi (1769-1795) who was an Italian naturalist and the first to describe a Mediterrean *Porphyra* species.

Description. In Northwest Atlantic specimens, blades are ovate to lanceolate, with slightly ruffled, irregular margins. Freshly collected blades are

light tan to greenish-brown, bronze to light green near holdfast, with their bases being slightly stipitate having small discoid holdfasts. Reproductively mature blades are 1-7 cm wide and 2.5-18 cm long; fronds monoecious with male gametangia forming distal streaks of various sizes extending through marginal female gametangia. Vegetative thalli 16-28.4 µm thick, with rectangular shaped cells 7.4-16 µm x 9.8-17.2 µm in surface view and 11.1-22.2 µm in transverse view. Each cell contains a single stellate chloroplast. Male gametangial portions of thalli 22.2-28.4 µm thick, with spermatangial packets 11.1-17.2 µm x 17.2-24.7 µm in surface view and 13.5-18.5 µm in transverse view containing 32 (64) spermatia arranged in 4 tiers of 8 (16) cells. Female gametangial areas 23.4 µm thick, with two trichogynes, one extending to each surface in transverse view. Zygotospores 4.9 µm x 9.8 µm in surface view; 12.3µm tall in transverse view arranged in 2 tiers of 4 in a packets, 9.7µm x 19.7 µm in surface view and 24.7 µm in transverse view.

Morphology and Cytology. Freshly collected fronds range in color from light tan to greenish-beige (L: 69.18, a:7.25, b:33.32) in vegetative areas and bronze to light green (L:70.56, a: -2.39, b:27.37) in the stipe area (Figure 4.1H-K & Table 4.1). Light tan colored male gametangia occur in streaks amongst light rose colored zygotosporangia near the distal half of blades. Thallus shape is highly variable, ranging from ovate to lanceolate with slightly ruffled, irregular margins (Figures 4.1H-K & 4.2A). The bases are slightly stipitate and have small discoid holdfast. Blade size varies from 2.5-18 cm in length (mean 6.04

±3.73SD) to 1-7 cm in width (mean 2.79 ±1.45SD) and tend to be slightly longer and narrower than Mediterranean specimens (Brodie et al. submitted).

The monostromatic blades ranges from 16-28.4 µm thick (mean 22.09 ±3.59SD) in vegetative areas; they are 22.2-28.4 µm thick (mean 23.09 ±2.34SD) in male gametangial areas, and 23.4 µm thick in female gametangial areas. Less than one third of specimens collected were reproductive with only a single specimen having both male and female gametangia. Vegetative cells have a single stellate chloroplast and are irregular shaped; dimensions are 7.4-16 µm (mean 11.38 ±2.65SD) x 9.8-17.2 µm (mean 13.49 ±2.58SD) in surface view and 11.1-22.2 µm (mean 14.31 ±2.76SD) in transverse view (Figure 4.2A-B). Spermatia are 2.4-4.9 µm (mean 4.91 ±1.41SD) x 3.7-4.9 µm (mean 4.03±.94SD) in surface view; 3.7-4.9 µm (mean 4.21 ±.64SD) in transverse view. Spermatangial packets contain 32 (64) spermatia that are arranged in 4 tiers of 8 (16) cells; the packets are 11.1-17.2 µm (mean 13.19 ±2.09SD) x 17.2-24.7 µm (mean 20.59 ±3.28SD) in surface view and 13.5-18.5 µm (mean 16.17 ±1.80SD) in transverse view (Figure 4.2C-D). In transverse view the female gametangia produce two trichogynes, one extending to each surface (Figure 4.2F). Zygotospores are 4.9 µm x 9.8µm in surface view and 12.3µm in transverse view. Zygotospores are arranged in 2 tiers of 4 within packets that are 9.7µm x 19.7 µm in surface view and 24.7 µm in transverse view (Figure 4.2 E-F).

Seasonality and Habitat. In New England blades were collected from January through April with all reproductive specimens collected in April. Most

specimens were found as epiphytes on *Fucus, Chondrus, Gracilaria,* and *Dumontia* within the mid to shallow subtidal.

Distribution. In New England *Porphyra olivii* is recorded from mid-Maine to Long Island Sound (Figure 4.3), occurring at two sites in Maine, one site in New Hampshire, five sites in Massachusetts, one site in Rhode Island, and three sites in Connecticut.

Molecular. Partial sequences of the *rbc*L, SSU, and ITS1 have been submitted to GenBank (Appendix C). No variation in *rbc*L, SSU, and ITS1 sequences was found within or among New England populations. Partial SSU sequences of New England populations were identical to Mediterranean populations; however, the *rbc*L sequences of New England populations differed from Mediterranean populations by a single transition at position 1413 (T vs C), while ITS1 sequences varied by 3-4 bp (Brodie et al., submitted).

Porphyra tsengii Mathieson, Bray, & Neefus *sp. nov.*

Holotype. NHA77786 (GenBank DQ813607, DQ813567, DQ813586). Collected at the New Meadows River, Brunswick, Maine, USA (43°54'40.12"N, 69°52'06.42"W), 12 March, 2004, coll. T. L. Bray & A. C. Mathieson, shallow subtidal on *Fucus*.

Isotypes. NHA77782, NHA77783, NHA77784, NHA77785 (GenBank DQ813585). Growing in the shallow subtidal on *Gracilaria* and *Fucus*.

Etymology. The name *Porphyra tsengii* commemorates the Chinese phycologist, C. K. Tsengii.

Description. Blade orbicular to lanceolate, base slightly stipitate with a small discoid holdfast, with irregular, multicellular protrusions along margins. Freshly collected blades light tan to greenish-brown. Reproductively mature blades 0.7-3.5 cm wide and 1.5-16 cm long. Monoecious with male gametangia forming distal streaks interrupting marginal female gametangial region. Vegetative areas of the thallus are 19.76-29.6 µm thick, irregular shaped cells 8.2-11.53 µm wide x 9.8-16.06 µm long in surface view and 12.35-18.53 µm high in transverse view. Vegetative cells contain a single stellate plastid. Male gametangial areas of the thallus are 22.23-29.64 µm thick, with spermatangial packets 11.1-17.29 µm wide x 22.23-27.1 µm long in surface view and 14.8-22.23 µm high in transverse view containing 32 spermatia arranged in 4 tiers of 8 cells. Female gametangial areas are 22.23-29.64 µm thick with zygotosporangial packets 11.12-17.29 µm wide x 12.35-17.29 µm long in surface view and 18.53-24.7 µm high in transverse view. Zygotospores are 4.94-7.41 µm wide x 4.94-7.41 µm long in surface view and 7.41-11.12 µm high in transverse view arranged in 2 tiers of 4.

Morphology and Cytology. Freshly collected fronds are light tan to beige (L:67.26, a:8.4, b:34.17) in vegetative area (Table 4.1). At distal half of blade, their lighter tan colored male gametangia occur in streaks within darker colored zygotosporangia. Thallus shape is variable from orbicular to lanceolate (Figure 4.1F-G). Margins are entire with irregular, multicellular protrusions and a stipitate base. Size of the blade is variable ranging from 1.5-16 cm in length (mean 5.1 ±4.29SD) to 0.7-3.5 cm (mean 2.66 ±2.33SD) in width.

Vegetative thalli are monostromatic with thickness ranging from 19.76-29.6 µm (mean 23.34 ±2.75SD). Vegetative cells contain a single stellate chloroplast. Cell dimensions range from 8.2-11.53 µm (mean 9.36 ±1.55SD) x 9.8-16.06 µm (mean 12.64 ±1.89SD) in surface view and 12.35-18.53 µm (mean 15.76 ±1.79SD) in transverse view (Figure 4.2G-H). Approximately half of the 10 specimens collected were reproductive, with 20% having both male and female reproductive structure on the same thallus, 20% only male gamatangia present, and 60% only zygotosporangia. Thalli containing male gametangia range in thickness from 22.23-29.64 µm (mean 25.94 ±5.24SD). In surface view spermatia range in size from 4.9-6.18 µm (mean 5.4±.91SD) x 4.9-6.18 µm (mean 5.4±.91SD) and 4.9-4.94 µm (mean 4.92 ±.03SD) in transverse view, with spermatia arranged in 4 tiers of 8 within packets. Spermatangial packets are 11.1-17.29 µm (mean 14.2 ±4.38SD) x 22.23-27.1µm (mean 24.67 ±3.44SD) in surface view and 14.8-22.23 µm (mean 18.52 ±5.25SD) in transverse view (Figure 4.2I-J). Areas of the thallus containing zygotosporangia are variable in thickness, ranging from 22.23-29.64 µm (mean 24.7 ±3.49SD). Zygotospores are also variable in size, ranging from 4.94-7.41 µm (mean 5.56 ±1.24SD) x 4.94-7.41 µm (mean 6.18±1.01SD) in surface view and 7.41-11.12 µm (mean 8.34 ±1.86SD) in transverse view. Zygotospores are arranged in 2 tiers of 4 within packets (Figure 4.2K-L). In surface view zygotosporangial packets range are 11.12-17.29 µm (mean 13.28±2.74SD) x 12.35-17.29 µm (mean 14.51±2.11SD) and 18.53-24.7 µm (mean 20.69 ±2.74SD) in transverse view.

Seasonality and Habitat. Specimens were collected during March and April from one estuarine tidal rapids site with a tidal gate that restricts the occurrence of low tide to approximately one quarter of its tidal amplitude. Thalli grow epiphytically on *Fucus* within the mid-low tidal zone (Table 4.1).

Distribution. Known from only one site in the New Meadows River, near Brunswick, ME (Figure 4.3). Collections at this site have occurred in multiple years (Appendix C).

Molecular. Partial sequences of the *rbc*L, SSU, and ITS1 have been submitted to GenBank (*rbc*L: NHA77785 GenBank DQ813585, NHA77786 GenBank DQ813607, NHA77792 GenBank DQ813608, NHA78066 GenBank DQ813609; SSU: NHA77786 GenBank DQ813586; ITS1: NHA77786 GenBank DQ813567, NHA77792 GenBank DQ813568). No variation in *rbc*L, SSU, and ITS1 sequences was found within or among populations.

Delineation. Unlike *Porphyra olivii, P. tsengii* is presently known from a very limited distribution (e.g. New Meadows River, Brunswick, Maine). While *P. olivii* has been collected from both mid- and sub-littorial elevations in a wide range of habitats (i.e. open coastal to tidal rapids), *P. tsengii* has only been collected from the mid-littorial in an estuarine tidal rapid. Additionally, the fresh, vegetative blades *P. tsengii* are mostly beige vs. the greenish, bronze color of *P. olivii* blades and tend to be more narrow (Table 4.1).

Porphyra stamfordensis Neefus, Bray, & Mathieson *sp. nov.*

Holotype. NHA78113 (GenBank DQ813641). Collected at Cove Island, Stamford, Connecticut, USA (41°02'31.08"N, 73°30'04.71"W) 18 December, 2004, coll. C. D. Neefus & A. K. Neefus, high littoral, epilithic.

Isotypes. NHA78158, NHA78159, NHA78160, NHA78161, NHA78162, NHA78163.

Eytmology. The name *Porphyra stamfordensis* refers to the type location, Stamford, Connecticut, USA.

Description. Blades monostromatic, orbicular to lanceolate in shape and frequently lacineate. Margins slightly ruffled with multicellular irregular protrusions. Color of freshly collected blades light to dark tan and greenish-bronze near holdfast. Base slightly stipitate with a small, discoid holdfast. Reproductively mature blades 3.5-20.5 cm long and 1-7.5 cm wide, monoecious with male gametangia forming variable distal streaks extending through marginal female gametangia. Vegetative areas of the thallus 24.7-32.1 µm thick, containing barrel-shaped cells 6.1-19.7 µm wide x 6.1-19.7 µm long in surface view and 12.3-22.2 µm high in transverse view with a single stellate chloroplast. Male gametangial areas 27.1-49.4 µm thick with spermatangial packets 12.3-19.7 µm wide x 17.2-30.8 µm long in surface view and 16.0-32.1 µm high in transverse view, containing 32 to 64 spermatia arranged as 4 or 8 tiers of 8 cells. Female gametangial areas 29.6-37 µm thick. Female gametes with two trichogynes extending to both surfaces. Zygotospores 3.7-7.4 µm wide x 3.7-9.8 µm long in surface view and 12.3µm high in transverse view; zygotospores

arranged in 2 tiers of 4 within packets 14.8 µm wide x 16.0 -19.7 µm long in surface view and 12.3 µm high in transverse view.

Morphology and Cytology. Freshly collected fronds are light to dark tan (L:63.9, a:.41, b:30.94) in vegetative areas, except near the greenish-bronze holdfast area (Figure 4.1C-E & Table 4.1), but they turn mauve to grayish purple with time after drying. Light tan colored male gametangia occur in distal streaks within deep rose colored zygotosporangial areas. Thallus shape is variable from orbicular to lanceolate and frequently lacineate. Blade margins are mostly entire and slightly ruffled with irregular, multicellular proliferations in vegetative areas (Figure 4.4A). Base of frond is stipitate and has a small, discoid holdfast. Frond size is variable from 3.5–20.5 cm long (mean 7.55 ±4.26SD) x 1–7.5 cm wide (mean 3.57 ±1.83SD). Thallus adheres well to herbarium paper.

Monostromatic fronds are 24.7-32.1 µm thick in transverse view (Figure 4.4B). Vegetative cells have a single stellate chloroplast (Figure 4.4A) and in surface view are barrel-shaped, range in size from 6.1-19.7 µm (mean 9.64 ±3.10SD) x 6.1-19.7 µm (mean 10.93 ±3.29SD); in transverse view they are 12.3-22.2 µm (mean 14.83 ±2.87SD) high (Table 4.1). Vegetative specimens comprised 68% of the 17 specimens collected with 22 % containing only male gametangia and 10% both male and female gametangia. Thallus thickness in male gametangial regions range from 27.1-49.4 µm (mean 33.52 ±8.66SD), with packet size 12.3-19.7 µm (mean 14.77 ±3.9SD) x 17.2-30.8 µm (mean 22.38 ±6.12SD) in surface view and 16-32.1 µm (mean 22.77 ±6.02SD) in transverse view (Table 4.1). Packets contain 32 (64) spermatia arranged as 4 (8) tiers of 8

cells (Figure 4.4C-D). Spermatia, which are clear in color, range in size from 2.4-7.4 µm (mean 4.7 ±1.68SD) x 3.7-9.8 µm (mean 5.93±1.68SD) in surface view and 2.4-4.9 µm (mean 3.65 ±1.37SD) in transverse view. Female gametangial areas of thalli range from 29.6-37 µm (mean 33.3 ±5.23SD) thick; in transverse view trichogynes extending to both surfaces (Figure 4.4F). Zygotosporangial packets range in size from 14.8 µm (mean 14.8 ±0.0SD) x 16-19.7 µm (mean 17.85±2.62SD) in surface view (Figure 4.4E) and 22.2-24.7 µm (mean 23.45 ±1.77SD) in transverse view. Packets are arranged as 2 tiers of 4 zygotospores that range in size from 3.7-7.4 µm (mean 5.55 ±2.62SD) x 3.7-9.8 µm (mean 6.75±4.31SD) in surface view and 12.3µm (mean 12.3 ±0.0SD) in transverse view.

Seasonality and Habitat. Collected from November through February with reproductive specimens appearing in January and February. Individuals grow either epilithically or epiphytically (on *Fucus*) within the mid to high intertidal zones. Collection sites include semi-exposed coastal regions as well as in tidal rapids areas.

Distribution. Specimens have been confirmed at six locations (Figure 4.3), two of which were in Massachusetts and four in Connecticut. No specimens were found north of Cape Cod.

Molecular. Partial sequences of the *rbc*L, SSU, and ITS1 have been submitted to GenBank (*rbc*L: NHA78113 GenBankDQ813641, NHA77883 GenBankDQ813636, NHA77921 GenBankDQ813640, NHA77888 GenBank DQ813637, NHA77895 GenBankDQ813638, NHA77901 GenBank DQ813639,

NHA78140 GenBankDQ813642; SSU: NHA77883 GenBankDQ813591, NHA77901 GenBankDQ813592; ITS1: NHA77883 GenBankDQ813577, NHA77901 GenBankDQ813578). No variation in *rbc*L, SSU, and ITS1 sequences was found within or among populations.

Delineation. Vegetative blades of *Porphyra stamfordensis* tend to be thicker and darker in color than *P. olivii* and *P. tsengii* (Table 4.1). Both *P. olivii* and *P. tsengii* occur mostly as epiphytes in the mid to sub-littorial, while *P. stamfordensis* is frequently found attached to rocks in the mid to upper littorial.

Porphyra spatulata Bray, Mathieson, & Neefus *sp. nov.*

Holotype. NHA77916 (GenBankDQ813632). Collected at Westport, Massachusetts, USA (N41°30'53.34",W 71°04'07.53") 8 February, 2005, coll. T. L. Bray & J. P. Day, subtital.

Isotype. NHA77917 (GenBankDQ813634), NHA77919 (GenBankDQ813576).

Etymology. The name *Porphyra spatulata* reflects the spatulate shape of the frond.

Description. Blades spatulate to obovate tapering to point at base with deeply ruffled margins extending entire length of blade. Freshly collected blade light tan to orange-beige. Reproductively mature blades 7.5-19.5 cm long, 2-4.5 cm wide. Fronds monoecious with male gametangia forming pale, yellow-beige marginal zone at distal half of blade interspersed with granular patches of deep rose-colored female gametangia. Vegetative areas of the thallus 17.2-24.7 μm

thick, with rectangular cells 9.8-13.5 µm wide x 9.8-14.8 µm long in surface view and 11.1-17.2 µm high in transverse view. Cells contain a single stellate chloroplast. Male gametangial areas are 22.2-28.4 µm thick, with spermatangial packets 12.3-19.7 µm wide x 17.2-24.7 µm long in surface view and 17.2-27.1 µm high in transverse view. Spermatangia contain 32 spermatia arranged as 4 tiers of 8 cells. Female gametangial areas 19.7 µm thick. Zygotospores 4.9-6.1 µm wide x 4.9-6.1 µm long in surface view, 4.9-8.6 µm high in transverse view, zygotosporangial packets 9.8-14.8 µm wide x 14.8-17.2 µm long in surface view and 14.8 µm-17.2 µm high in transverse view containing 8 zygotospores arranged as 2 tiers of 4.

Morphology and Cytology. Freshly collected fronds are peach to orange-beige (L:72.36, a:7.28, b:31.66) in vegetative area. Male gametangia form a light tan marginal zone at the distal half of blade, which is separated from vegetative areas by granular patches of red zygotosporangia. Thallus shape is oblanceolate to obovate, with numerous deep ruffles along the entire length of the blade (Figure 4.1A-B). Base of frond is visibly stipitate with a long, slender stipe. Blade size ranges from 7.5-19.5 cm long (mean 11.94 ±4.59SD) to 2-4.5 cm wide (mean 3.44 ±1.21SD). Dried specimens adhere extremely well to herbarium paper and turn deep mauve with age.

In transverse view, the monostromatic, vegetative thalli range from 17.2-24.7 µm (mean 22.36 ±3.29SD) in thickness. In surface view their rectangular vegetative cells, which contain a single, stellate chloroplast are 9.8-13.5 µm (mean 11.51 ±1.61SD) x 9.8-14.8 µm (mean 12.15 ±1.54SD) in surface view and

11.1-17.2 µm (mean 13.66 ±2.00SD) in transverse view (Table 4.1 & Figure 4.4G-H). All specimens collected (5) were reproductive, with 62.5% containing only male gametangia, 25% only female gametangia, and 12.5% both male and female gametangia. Thallus thickness in male gametangial regions range from 22.2-28.4 µm (mean 33.52 ±2.17SD). Spermatia are 2.4-4.9 µm (mean 4.07 ±1.29SD) x 2.4-6.1 µm (mean 4.88±1.35SD) in surface view vs. 2.4-4.9 µm (mean 4.07 ±1.29SD) in transverse view. Spermatangial packets contain 32 spermatia arranged in 4 tiers of 8 and range in size from 12.3-19.7 µm (mean 14.37 ±2.89SD) x 17.2-24.7 µm (mean 20.77 ±3.18SD) in surface view by 17.2-27.1 µm (mean 20.73 ±3.53SD) in transverse view (Table 4.1 & Figure 4.4I-J). Thallus thickness in female gametangial regions was 19.7 µm, with zygotospores ranging in size from 4.9-6.1 µm (mean 5.7 ±.69SD) x 4.9-6.1 µm (mean 5.3±.69SD) in surface view and 4.9-8.6 µm (mean 6.97 ±1.89SD) in transverse view. Zygotospores were arranged in packets of 8 in 2 tiers of 4, with packets 9.8-14.8 µm (mean 12.7 ±2.59SD) x 14.8-17.2 µm (mean 15.6±1.39SD) in surface view and 14.8-17.2 µm (mean 16.4 ±1.39SD) in transverse view (Figure 4.4 K-L).

Seasonality and Habitat. Collected from February to April, specimens were found as drift material within shallow embayments and growing on shallow subtidal rocks in tidal rapids.

Distribution. Known from only two locations in Massachusetts (Figure 4.3). The more northern site (Duxbury, MA) is a protected shallow embayment

where only drift material was found, while at the more southern site (Westport, MA) individuals were growing on rocks within the subtidal.

Molecular. Partial sequences of the *rbc*L, SSU, and ITS1 have been submitted to GenBank (*rbc*L: NHA77916 GenBankDQ813632, NHA78031 GenBankDQ813635, NHA78032 GenBankDQ813633, NHA77917 GenBankDQ813634; SSU: NHA77916 GenBankDQ813589, NHA78032 GenBankDQ813590; ITS1: NHA77919 GenBankDQ813576). No variation in *rbc*L, and SSU sequences was found within or among populations.

Delineation. *Porphyra spatulata* can be destinguished from the previously described species (*P. olivii*, *P. tsengii*, and *P. stamfordensis*) based upon blade shape (oblanceolate), color (peach to orange-beige) and margins (extremely ruffled). In addition, reproductive blades of *P. spatulata* form male gametangia in marginal zones rather than streaks or patches. Among species described in this study, *P. spatulata* is the only taxa to be collected exclusively from the sub-littorial or as drift material.

Porphyra collinsii Neefus, Mathieson & Bray *sp. nov.*

Holotype. NHA78139 (GenBankDQ815594). Collected at Millstone, Connecticut, USA (41°18'15.38"N, 72°09'55.25"W) 23 April, 2004, coll. A. C. Mathieson, mid-low littoral.

Isotype. NHA78157 (GenBankDQ813593).

Etymology. The name *Porphya collinsii* commemorates Frank Shipley Collins for his extensive studies of New England seaweeds.

Description. Blades orbicular to lanceolate, with slightly ruffled margins. Freshly collected blades are light tan to pinkish beige, with a slightly stipitate base and small discoid holdfast. Reproductively mature blades 0.6-15.5 cm wide and 3.5-21 cm long; fronds monoecious with male gametangia forming a "speckled" or "splash" pattern of various size distal streaks or patches within marginal female gametangia. Vegetative portion of the thalli 17.6-27.1 µm thick, with rectangular shaped cells 7.4-17.2 µm wide x 9.8-22.2 µm long in surface view and 8.6-20.99 µm high in transverse view. Vegetative cells with a single stellate chloroplast. Male gametangial areas 19.7-27.7 µm thick, with spermatangial packets 9.88-17.2 µm wide x 17.2-27.1 long µm in surface view and 17.2-24.7 µm high in transverse view, containing 32 spermatia arranged in 4 tiers of 8 cells. Female gametangial areas 24.7-32.1 µm thick, with trichogynes extending to both surfaces. Zygotospores 4.9–12.35 µm wide x 7.4-12.35 µm long in surface view; 7.4-11.2 µm high in transverse view. Zygotosporangial packets 12.3-24.7 µm wide x 17.2-27.17 µm long in surface view and 17.2-22.23 µm tall in transverse view, containing 8 zygotospores arranged as 2 tiers of 4.

Morphology and Cytology. Freshly collected fronds range in color from light tan to pinkish-beige (L: 71.02, a:4.12, b:28.63) in vegetative areas. Light tan colored male gametangia occur in streaks and patches amongst light rose colored zygotosporangia near the distal half of blades. Thallus shape is variable, ranging from orbicular to lanceolate with slightly ruffled margins (Figure 4.5G-I). The bases are slightly stipitate and have small discoid holdfast. Blade size

varies from 3.5-21 cm in length (mean 6.04 ±3.73SD) to 0.6-15.5 cm in width (mean 2.79 ±1.45SD).

Blades are monostromatic and ranges from 17.6-27.17 µm thick (mean 23.18 ±3.69SD) in vegetative areas; they are 19.7-27.7 µm thick (mean 25.19 ±2.49SD) in male gametangial areas, and 24.7-32.1 µm thick (mean 27.99 ±3.77SD) in female gametangial areas. Most specimens examined were reproductive (71%) with 9 specimens having only male gametangia, a single specimen having only female gametangia, and 2 specimens with both male and female gametangia. Vegetative cells, which have a single stellate chloroplast are 7.4-17.2 µm (mean 12.08 ±3.29SD) x 9.8-22.2 µm (mean 14.93 ±3.61SD) in surface view; they are also 8.6-20.99 µm (mean 15.17 ±4.42SD) in transverse view (Figure 4.5 A-B). Spermatia are 4.9-7.4 µm (mean 6.27 ±1.50SD) x 3.7-4.9 µm (mean 4.25±.94SD) in surface view; 3.7-4.9 µm (mean 4.58 ±0.56SD) in transverse view. Spermatangial packets contain 32 spermatia that are arranged in 4 tiers of 8 cells; the packets are 9.88-17.2 µm (mean 13.44 ±2.23SD) x 17.2-27.1 µm (mean 21.44 ±3.10SD) in surface view and 17.2-24.7 µm (mean 21.08 ±2.4SD) in transverse view (Figure 4.5C-D). Zygotospores are 4.9-12.35 µm (mean 7.78 ±4.00SD) x 7.4-12.35 µm (mean 9.05 ±2.86SD) in surface view and 7.4-11.12 µm (mean 9.44 ±1.89SD) in transverse view . Zygotospores are arranged in 2 tiers of 4 within packets that are 12.3-24.7 µm (mean 17.27 ±6.56SD) x 17.2-27.17 µm (mean 22.19 ±4.99SD) in surface view and 17.2-22.23 µm (mean 19.71 ±2.49SD) in transverse view (Figure 4.5E-F).

Seasonality and Habitat. Blades were collected from January through April. Most specimens were found as epiphytes on *Chondrus, Gracilaria,* and *Dumontia* within the mid to shallow subtidal zones, with two samples collected as drift.

Distribution. *Porphyra collinsii* is recorded from mid-Maine to Long Island Sound, with approximately half of the collection sites found north of Cape Cod and half in Long Island Sound (Figure 4.3).

Molecular. Partial sequences of the *rbc*L, SSU, and ITS1 have been submitted to GenBank (*rbc*L: NHA78139 GenBankDQ815594, NHA78157 GenBankDQ813593, NHA78005 GenBankDQ813617, NHA77861 GenBankDQ813595, NHA77862 GenBankDQ813596, NHA77864 GenBankDQ813597, NHA77878 GenBankDQ813598, NHA77955 GenBankDQ813599, NHA77957 GenBankDQ8136000, NHA77958 GenBankDQ813601, NHA78100 GenBankDQ813602, NHA78163 GenBankDQ813605, NHA78164 GenBankDQ813606, NHA8560 GenBankDQ813603, BM000806074 GenBankDQ813604; SSU: NHA78139 GenBankDQ813583, NHA77878 GenBankDQ813584; ITS1: NHA78139 GenBankDQ813565, NHA78005 GenBankDQ813574, NHA77878 GenBankDQ813566). No variation in *rbc*L, SSU, and ITS1 sequences was found within or among populations.

Delineation. Mature *Porphyra collinsii* blades tend to be larger than all other species described in this study and slightly more reddish in color. Most characteristic of this species is the "speckled" or "splash" pattern of the male

gametangia on the distal half of reproductive blades vs. distal streaks that mostly extend to the margins as in *P. olivii, P. tsengii,* and *P. stamfordensis.*

Sequence Comparisons

Within the context of all species of *Porphyra* currently recorded in the Northwest Atlantic, each of the five cryptic taxa are molecularly unique (Table 4.2 & Figure 4.6). Sequence data (*rbc*L, SSU, ITS1) from four of the five cryptic species (*Porphyra tsengii, P. stamfordensis, P. spatulata, P. collinsii*) were compared to GenBank sequences with no matches found. Morphological and ecological comparisons of these cryptic species were also made to the original descriptions of 15 species with similar reproductive structures (i.e. male gametangial patches or streaks) which do not have sequences on GenBank. However, based on the morphology and ecology the cryptic species remained distinctive.

Phylogenies

All three analyses (Bayesian, NJ, MP) generated trees with the same topology (Figure 4.6). The single most parsimonious tree generated by MP analysis was 534 steps in length, when *Smithora naiadum* was used as an outgroup. The consistency index of the MP tree, excluding uninformative characters, was 0.667 with a retention index of 0.678. The NJ tree had an ME score of 0.4580.

All three analyses generated trees in which every cryptic species was located within a strongly supported, monophyletic clade that also included *Porphyra leucosticta, P.yezoensis, P. rosengurttii,* and *P. katadae* (Figure 4.6). Divergence of *rbc*L sequences within this clade ranged from 0.8% (13 bp) between *P. collinsii* and *P. olivii* to 6.8% (69 bp) between *P. yezoensis* and *P. stamfordensis* (Table 4.2 & Figure 4.6). Divergence of SSU sequences within this clade ranged from 0.2% (1 bp) between *P. collinsii* and *P. olivii* to 3.6% (15 bp) between *P. tsengii* and *P. stamfordensis* (Table 4.2). With the exception of *P. spatulata* and *P. katadae*, all the members of this clade share the common morphological feature of male gametangia arranged in marginal "streaks" or "patches."

Additionally, members of this clade were distinct from currently recognized Northwest Atlantic species of *Porphyra* with divergences of *rbc*L sequences ranging from 3.1% (32 bp) between *P.collinsii* and *P. leucosticta* to 10.7% (108 bp) between *P. tsengii* and *P. purpurea*. Divergences of SSU sequences range from 2.7% (14 bp) between *P. olivii* and *P. leucosticta* to 13.4% (61 bp) between *P. tsengii* and *P. purpurea*.

Discussion

The suspicion that the circumscription of some species of *Porphyra* have been too broadly interpreted for the marine flora in the Northwest Atlantic is further substantiated by this study. Historically, *Porphyra* having male gametangia arranged in distal streaks or patches were identified as *P. leucosticta* (Bird & McLachlan 1992; Taylor 1957) Based on the arrangement of male

gametangia in marginal "streaks" or "patches" (Figures 4.1 & 4.5), four of the five cryptic taxa (*P. olivii, P. tsengii, P. stamfordensis, P. collinsii*) would have been morphologically confused in the northwestern Atlantic with either *P. leucosticta* or the recently introduced *P.yezoensis* Ueda (West et al. 2005). Additionally, sexually immature blades of the fifth cryptic species, *P. spatulata,* could be easily confused with the introduced Asiatic species, *P. katadae* (Chapter 3), although sexually mature specimens can be easily distinguished from one another (i.e. sexually non-sectored vs. sectored blade).

In all phylogenetic analyses, these cryptic species were arranged within the same well supported monophyletic clade (Figure 4.6). With the exception of *Porphyra katadae* and *P. spatulata*, this clade would seem to support a correlation between species having male gametangia arranged in streaks or patches and phylogenetic relationship. In the phylogenetic analyses of the order Bangiales using the SSU rRNA gene, Nelson et al. (2006) argued that the members of some clades of *Porphyra* can be consistently linked by morphological and anatomical characters. One such example given by Nelson et al. (2006) was a clade containing *P. yezoensis, P. leucosticta,* and *P. rakiura,* all of which share a very similar arrangement of gametangial regions of the blade (i.e. male gametangial streaks or patches). The affinity of such morphologically similar species as *P. leucosticta* and *P. yezoensis* has also been shown in other studies (Klein et al. 2003; Yamazaki et al. 1996). Additionally, it is interesting to note that these cryptic species showed a closer affinity to Pacific and Mediterranean species, *P. yezoensis, P. katadae, P. olivii,* and *P. rosengurttii*

than to (probably) endemic North Atlantic species, *P. miniata, P. amplissima, P. birdiae, P. umbilicalis, P. linearis,* and *P. purpurea* (Figure 4.6).

The lack of variability in the less conserved ITS1 region among geographically distinct populations of each cryptic taxa seems to support the recent introductions of these cryptic taxa. A lack of ITS1 sequence variation was also found among geographically distinct populations of two introduced strains of the Asiatic species, *P. yezoensis* and *P. katadae* occurring New England (Chapter 2). Likewise, Broom et al. (2002) reported a single ITS haplotype common to samples of *P. suborbiculata* from Connecticut and North Carolina and suggested that its occurrence in the Western Atlantic was of recent introduction. Conversely, among historically described species in the Northwest Atlantic ITS1 sequences have been shown to vary among geographically distinct populations (Bray et al. 2006, Stiller & Waaland 1996, Teasdale 2004).

Also suggestive of recent introductions is the circumscribed distributions of *P. tsengii*, *P. spatulata*, and *P. stamfordensis* (Figure 4.3). The distributional patterns of *P. spatulata*, and *P. stamfordensis* (Long Island Sound and Cape Cod Canal area) closely compare with the distributional patterns of two Asiatic introductions, *P. katadae* and *P. yezoensis* f. *narawaensis* (Chapters 2 & 3). The restriction of *P. tsengii* to one site on the New Meadows River near Brunswick, ME would suggest a common single source of introduction. In contrast to the other cryptic species, *P. olivii* has a broad distribution (Figure 4.3). However, the lack of ITS1 sequence variation across this wide distribution may suggest multiple introductions from a common source or the coastwise spread of a single

introduction by domestic shipping (Hines et al. 2004). A similar distributional pattern was reported for the introduced *P. yezoensis* f. *yezoensis* (Chapter 2).

The limited distribution of *P. spatulata* and *P. stamfordensis* occurs within major shipping routes (i.e. Long Island Sound) suggesting introductions may have been the result of ballast water discharge or hull fouling (Fofonoff et al. 2003, Carlton et al. 1995). However, another possible source of introduction within Long Island Sound is shellfish cultivation. Shellfish cultivation sites have long been suspect of being a major pathway in the introduction and establishment of exotic species (Siguan 2003, Verlaque 2001). Verlaque et al. (2005) reported that 80% of the introduced species found at a shellfish cultivation site in the Mediterranean (Thau Lagoon, France) were native to the two largest oyster exporting countries in the world, Japan and Korea. In Connecticut, Getchis (2005) reported that forty shellfish companies lease over 67,000 acres of Long Island Sound bottom. In Massachuesetts approximately 650 acres of tidelands are licensed for shellfish cultivation in 22 towns (http://www.mass.gov/czm/wpshell.htm); some adjacent to *P. spatulata*, *P. stamfordensis*, and *P. olivii* collection sites (*e.g.* Duxbury, Plymouth, Barnstable, Falmouth, Fairhaven). Currently, two aquaculture companies are cultivating American oysters (*Crassostrea virginica* Gmelin), European oysters (*Ostrea edulis* L.), and quahogs (*Mercenaria mercenaria* L.) immediately upstream from the solitary *P. tsengii* site on the New Meadows river near Brusnwick, ME (http://www.maine.gov/dmr/aquaculture/leaseinventory2005/newmeadowsriver.htm).

Conclusion

This study supports the argument of Brodie and Irvine (2003) that *Porphyra leuctosticta* could represents an "aggregate of species." Based upon historical herbarium specimens, four of the five species reported in this study as well as the recently reported *P. yezoensis* (Chapter 2) were historically reported in the Northwest Atlantic as *P. leucosticta.* The delineation of species based solely upon the morphological character of male gametangial appearing as streaks or patches is no longer definitive in the northwestern Atlantic, but instead will require a combination of molecular, morphological, and ecological characters as presented in this study.

While this study is not exhaustive, it does suggest the diversity of *Porphyra* in the Northwest Atlantic is much greater than has been previously thought. With the increase of inadvertent introductions worldwide via shipping and aquaculture, this diversity will no doubt continue to grow requiring a constant monitoring of local floras. The naming of new species in this study for taxa that appear to be non-native to the northwestern Atlantic accentuates the need for the accumulation of sequence data for *Porphyra* worldwide. With sequences submissions for ~ 60 known species and ~ 40 unknown species currently on GenBank, the possibility of taxonomic confusion continues to be a real threat.

Table 4.1. A comparison of morphology and ecology among *Porphyra olivii, P. tsengii, P. stamfordensis, P. spatulata,* and *P. collinsii.*

	Porphyra olivii	***Porphyra tsengii***	***Porphyra stamfordensis***	***Porphyra spatulata***	***Porphyra collinsii***
Blade Morphology					
Color	Light tan to greenish-beige (L: 69.18, a:7.25, b:33.32) in vegetative area; bronze to light green (L:70.56, a: -2.39, b:27.37) in stipe area.	Light tan to beige (L:67.26, a:8.4, b:34.17) in vegetative area.	Light to dark tan (L:63.9, a:.41, b:30.94) in vegetative area.	Peach to orange-beige (L:72.36, a:7.28, b:31.66) in vegetative area.	Light tan to pinkish beige (L:71.02, a:4.12, b:28.63) in vegetative areas.
Shape	Ovate to lanceolate	Orbicular to lanceolate	Orbicular to lanceolate	Obovate to oblanceolate	Orbicular to laceolate
Reproductive pattern	Light tan colored male gametangia occuring in streaks within light rose colored zygotosporangia at margins of blade.	Light tan colored male gametangia occuring in streaks among scattered, light pink clusters of zygotosporangia at distal half of blade.	Light tan colored male gametangia occuring in streaks within deep rose colored zygotosporangia at distal half of blade.	Light tan male gametangia forming marginal zone at distal half of blade separated from vegetative areas by granular patches of red zygotosporangia.	Light tan colored male gametangia occuring in speckled patches within light rose colored zygotosporangia at distal half of blade.
Margin	Entire; slightly ruffled; with irregular, multicellular protrusions	Entire; with irregular, multicellular protrusions	Entire, slightly ruffled, occasionally lacineate; with irregular, multicellular protrusions	Entire; extremely ruffled	Entire; slightly ruffled
Base	Stipitate	Stipitate	Stipitate	Stipitate	Stipitate

Table 4.1. Continued.

	Porphyra olivii	*Porphyra tsengii*	*Porphyra stamfordensis*	*Porphyra spatulata*	*Porphyra collinsii*
Dimensions	2.5-18 cm (mean 6.04 ±3.73SD) L x 1-7 cm (mean 2.79 ±1.45SD) W	1.5-16 cm (mean 5.1 ±4.29SD) L x .7-3.5 cm (mean 2.66 ±2.33SD) W	3.5-20.5 cm (mean 7.55 ±4.26SD) L x 1-7.5 cm (mean 3.57 ±1.83SD) W	7.5-19.5 cm (mean 11.94 ±4.59SD) L x 2-4.5 cm (mean 3.44 ±1.21SD) W	3.5-21 cm (mean 10.98 ±6.47SD) L x 0.6-15.5 cm (mean 5.84 ±3.81SD) W
Cell Morphology					
Vegetative					
Thallus Thickness	16-28.4 µm (mean 22.09 ±3.59SD)	19.76-29.6 µm (mean 23.34 ±2.75SD)	24.7-32.1 µm (mean 26.12 ±3.03SD)	17.2-24.7 µm (mean 22.36 ±3.29SD)	17.6-27.17 µm (mean 23.18 ±3.69SD)
Cell Layers	one	one	one	one	one
Chloroplast	single, stellate	single, stellate	single, stellate	single, stellate	single, stellate
Cell Dimensions	7.4-16 µm (mean 11.38 ±2.65SD) x 9.8-17.2 µm (mean 13.49 ±2.58SD) in surface view; 11.1-22.2 µm (mean 14.31 ±2.76SD) in transverse view.	8.2-11.53 µm (mean 9.36 ±1.55SD) x 9.8-16.06 µm (mean 12.64 ±1.89SD) in surface view; 12.35-18.53 µm (mean 15.76 ±1.79SD) in transverse view.	6.1-19.7 µm (mean 9.64 ±3.10SD) x 6.1-19.7 µm (mean 10.93 ±3.29SD) in surface view; 12.3-22.2 µm (mean 14.83 ±2.87SD) in transverse view.	9.8-13.5 µm (mean 11.51 ±1.61SD) x 9.8-14.8 µm (mean 12.15 ±1.54SD) in surface view; 11.1-17.2 µm (mean 13.66 ±2.00SD) in transverse view.	7.4-17.2 µm (mean 12.08 ±3.29SD) x 9.8-22.2 µm (mean 14.93 ±3.61SD) in surface view; 8.6-20.99 µm (mean 15.17 ±4.42SD) in transverse view.
Male Gamatangia					
Thallus Thickness	22.2-28.4 µm (mean 23.09 ±2.34SD)	22.23-29.64 µm (mean 25.94 ±5.24SD)	27.1-49.4 µm (mean 33.52 ±8.66SD)	22.2-28.4 µm (mean 33.52 ±2.17SD)	19.7-27.17 µm (mean 25.19 ±2.49SD)
Arrangement	4 tiers of 8 (16)	4 tiers of 8	4(8) tiers of 8	4 tiers of 8	4 tiers of 8
Packet Dimensions	11.1-17.2 µm (mean 13.19 ±2.09SD) x 17.2-24.7 µm (mean 20.59 ±3.28SD) in surface view; 13.5-18.5 µm (mean 16.17 ±1.80SD) in transverse view	11.1-17.29 µm (mean 14.2 ±4.38SD) x 22.23-27.1µm (mean 24.67 ±3.44SD) in surface view; 14.8-22.23 µm (mean 18.52 ±5.25SD) in transverse view.	12.3-19.7 µm (mean 14.77 ±3.9SD) x 17.2-30.8 µm (mean 22.38 ±6.12SD) in surface view; 16-32.1 µm (mean 22.77 ±6.02SD) in transverse view.	12.3-19.7 µm (mean 14.37 ±2.89SD) x 17.2-24.7 µm (mean 20.77 ±3.18SD) in surface view; 17.2-27.1 µm (mean 20.73 ±3.53SD) in transverse view	9.88-17.2 µm (mean 13.44 ±2.23SD) x 17.2-27.1 µm (mean 21.44 ±3.10SD) in surface view; 17.2-24.7 µm (mean 21.08 ±2.4SD) in transverse view.

Table 4.1. Continued.

	Porphyra olivii	*Porphyra tsengii*	*Porphyra stamfordensis*	*Porphyra spatulata*	*Porphyra collinsii*
Cell Dimensions	2.4-4.9µm (mean 4.91 ±1.41SD) x 3.7-4.9 µm (mean 4.03±.94SD) in surface view; 3.7-4.9 µm (mean 4.21 ±.64SD) in transverse view.	4.9-6.18 µm (mean 5.4±.91SD) x 4.9-6.18 µm (mean 5.4±.91SD) in surface view; 4.9-4.94 µm (mean 4.92 ±.03SD) in transverse view.	2.4-7.4 µm (mean 4.7 ±1.68SD) x 3.7-9.8 µm (mean 5.93±1.68SD) in surface view; 2.4-4.9 µm (mean 3.65 ±1.37SD) in transverse view.	2.4-4.9 µm (mean 4.07 ±1.29SD) x 2.4-6.1 µm (mean 4.88±1.35SD) in surface view; 2.4-4.9 µm (mean 4.07 ±1.29SD) in transverse view.	4.9-7.4 µm (mean 6.27 ±1.50SD) x 3.7-4.9 µm (mean 4.25±0.85SD) in surface view; 3.7-4.9 µm (mean 4.58 ±0.56SD) in transverse view.
Zygotosporangia					
Thallus Thickness	23.4 µm	22.23-29.64 µm (mean 24.7 ±3.49SD)	29.6-37 µm (mean 33.3 ±5.23SD)	19.7 µm	24.7-32.1 µm (mean 27.99 ±3.77SD)
Arrangement	2 tiers of 4	2 tiers of 4	2 tiers of 4	2 tiers of 4	2 tiers of 4
Packet Dimensions	9.7µm x 19.7 µm in surface view; 24.7 µm in transverse view.	11.12-17.29 µm (mean 13.28±2.74SD) x 12.35-17.29 µm (mean 14.51±2.11SD) in surface view; 18.53-24.7 µm (mean 20.69 ±2.74SD) in transverse view.	14.8 µm (mean 14.8 ±0.0SD) x 16-19.7 µm (mean 17.85±2.62SD) in surface view; 22.2-24.7 µm (mean 23.45 ±1.77SD) in transverse view.	9.8-14.8 µm (mean 12.7 ±2.59SD) x 14.8-17.2 µm (mean 15.6±1.39SD) in surface view; 14.8-17.2 µm (mean 16.4 ±1.39SD) in transverse view.	12.3-24.7 µm (mean 17.27 ±6.56SD) x 17.2-27.17 µm (mean 22.19±4.99SD) in surface view; 17.2-22.23 µm (mean 19.71 ±2.49SD) in transverse view.
Cell Dimensions	4.9 µm x 9.8µm in surface view; 12.3µm in transverse view.	4.94-7.41 µm (mean 5.56 ±1.24SD) x 4.94-7.41 µm (mean 6.18±1.01SD) in surface view; 7.41-11.12µm (mean 8.34 ±1.86SD) in transverse view.	3.7-7.4 µm (mean 5.55 ±2.62SD) x 3.7-9.8 µm (mean 6.75±4.31SD) in surface view; 12.3µm (mean 12.3 ±0.0SD) in transverse view.	4.9-6.1 µm (mean 5.7 ±.69SD) x 4.9-6.1 µm (mean 5.3±.69SD) in surface view; 4.9-8.6 µm (mean 6.97 ±1.89SD) in transverse view	4.9-12.35 µm (mean 7.78 ±4.00SD) x 7.4-12.35 µm (mean 9.05±2.86SD) in surface view; 7.4-11.12 µm (mean 9.44 ±1.89SD) in transverse view

Table 4.1. Continued.

	Porphyra olivii	*Porphyra tsengii*	*Porphyra stamfordensis*	*Porphyra spatulata*	*Porphyra collinsii*
Ecology					
Seasonality	Winter-Spring	Spring	Fall -Spring	Spring	Winter-Spring
Elevation	Mid-sub littorial	Mid-littorial	High-mid littorial	Sub-littorial	Mid-sub littorial
Substrata	*Fucus, Chondrus, Gracilaria, Dumontia*	*Fucus*	rock; *Fucus*	Drift and rock	*Chondrus, Gracilaria, Dumontia,* and drift
Habitat	Open coasts, embayments, and tidal rapids	Estuarine tidal rapids	Open coast and estuarine tidal rapids	Shallow embayments and estuarine tidal rapids	Open coasts, embayments, and tidal rapids

Table 4.2. Percent divergence between *rbc* L and SSU sequences from cryptic and introduced taxa in the Northwest Atlantic. Diagnonal line and below = partial SSU exon (426 bp); above diagonal line = partial *rbc* L and partial *rbc* L-*rbc* S spacer (1077 bp from position 390 of *rbc* L to position 27 of the *rbc* L-*rbc* S spacer). n/a = sequence not available.

		***rbc* L**								
		P. tsengii	*P. olivii*	*P. collinsii*	*P. yezoensis* f. *yezoensis*	*P. yezoensis* f. narawaensis	*P. rosengurttii*	*P. spatulata*	*P. katadae*	*P. stamfordensis*
SSU	*P. tsengii*		2.4	2.3	2.2	2.1	2.8	4.6	4.8	6.1
	P. olivii	2.4		0.8	2.7	2.6	3.0	5.1	4.9	6.1
	P. collinsii	2.6	0.2		2.6	2.5	3.1	4.9	4.8	5.7
	P. yezoensis f. *yezoensis*	n/a	n/a	n/a		0.1	3.3	5.5	5.5	6.8
	P. yezoensis f. narawaensis	2.6	2.2	2.4	n/a		3.2	5.4	5.4	6.6
	P. rosengurttii	n/a	n/a	n/a	n/a	n/a		5.3	4.9	6.6
	P. spatulata	3.1	2.6	2.9	n/a	1.4	n/a		1.7	4.0
	P. katadae	2.6	1.7	1.4	n/a	1.4	n/a	1.4		4.2
	P. stamfordensis	3.6	2.6	2.4	n/a	1.9	n/a	1.4	1.4	

Figure 4.1. Blade morphology of *Porphyra spatulata* (A-B), *P. stamfordensis* (C-E), *P. tsengii* (F-G), and *P. olivii* (H-K).

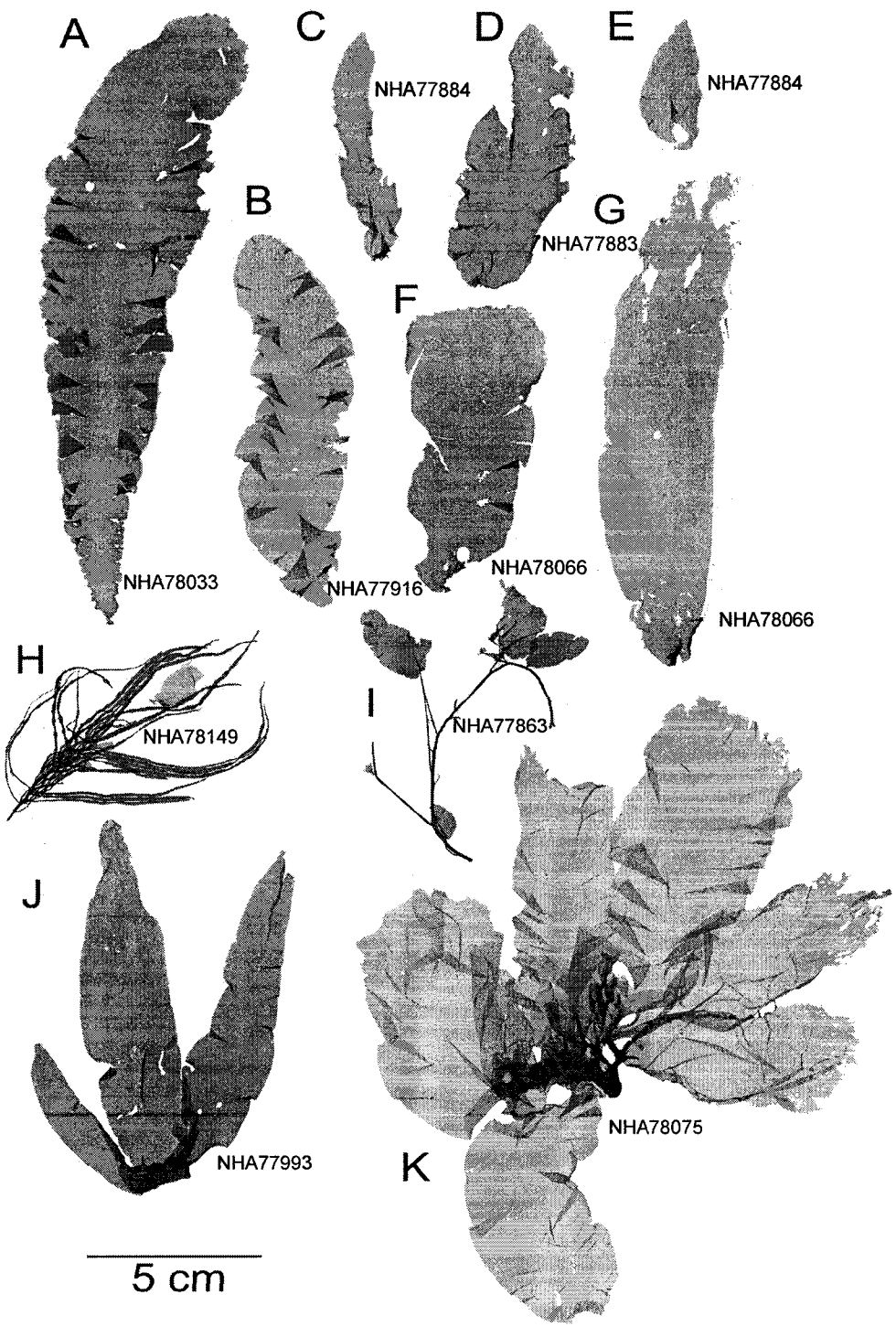


Figure 4.2. Surface and tranverse views of vegetative, male gametangial, and female gametangial thallus regions of *Porphyra olivii* NHA77994 (A-F) and *P. tsengii* NHA78066 (G-L).

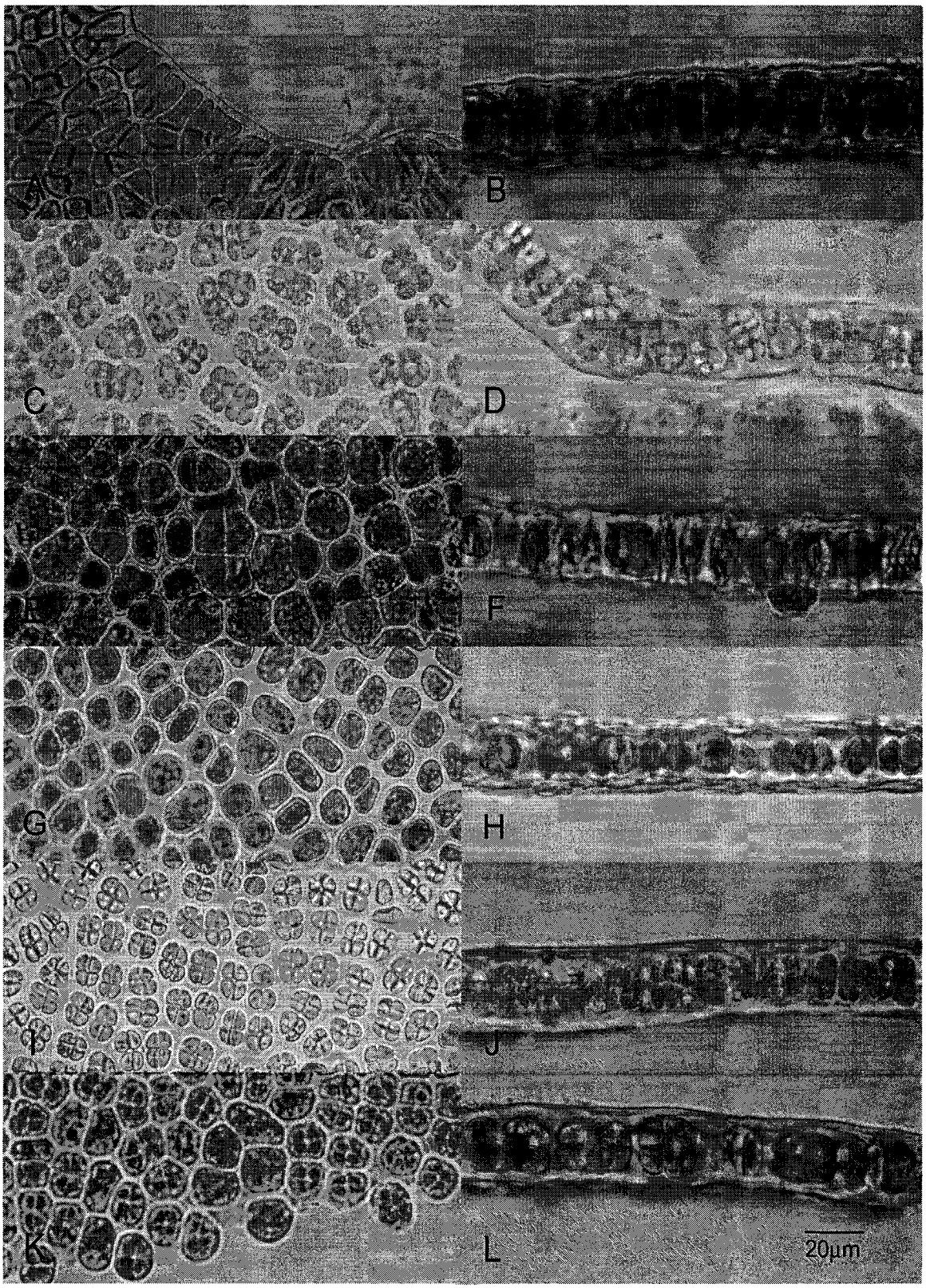


Figure 4.3. Distributional patterns of *Porphyra stamfordensis, P. spatulata, P. olivii, P. collinsii* and *P. tsengii* in the Northwest Atlantic.

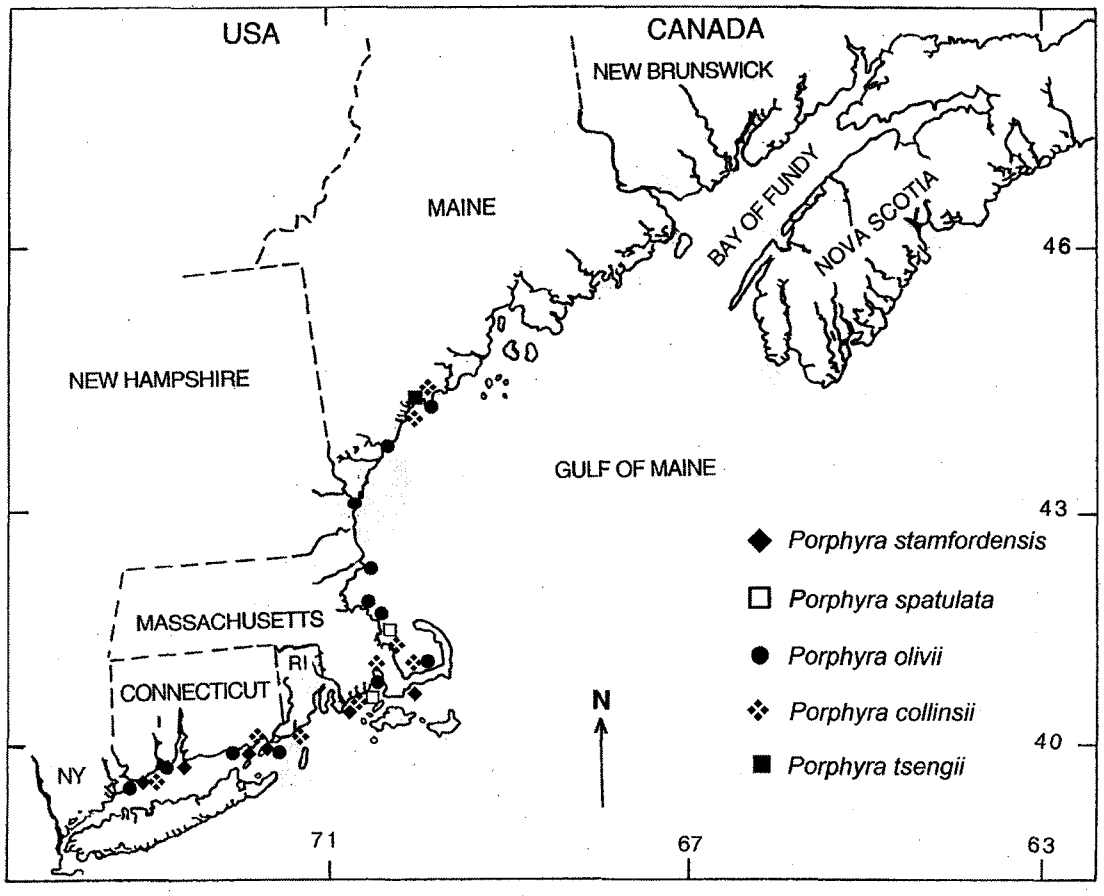


Figure 4.4. Surface and tranverse views of vegetative, male gametangial, and female gametangial thallus regions of *Porphyra stamfordensis* NHA78066 (A-F) and *P. spatulata* NHA78032 (G-L).

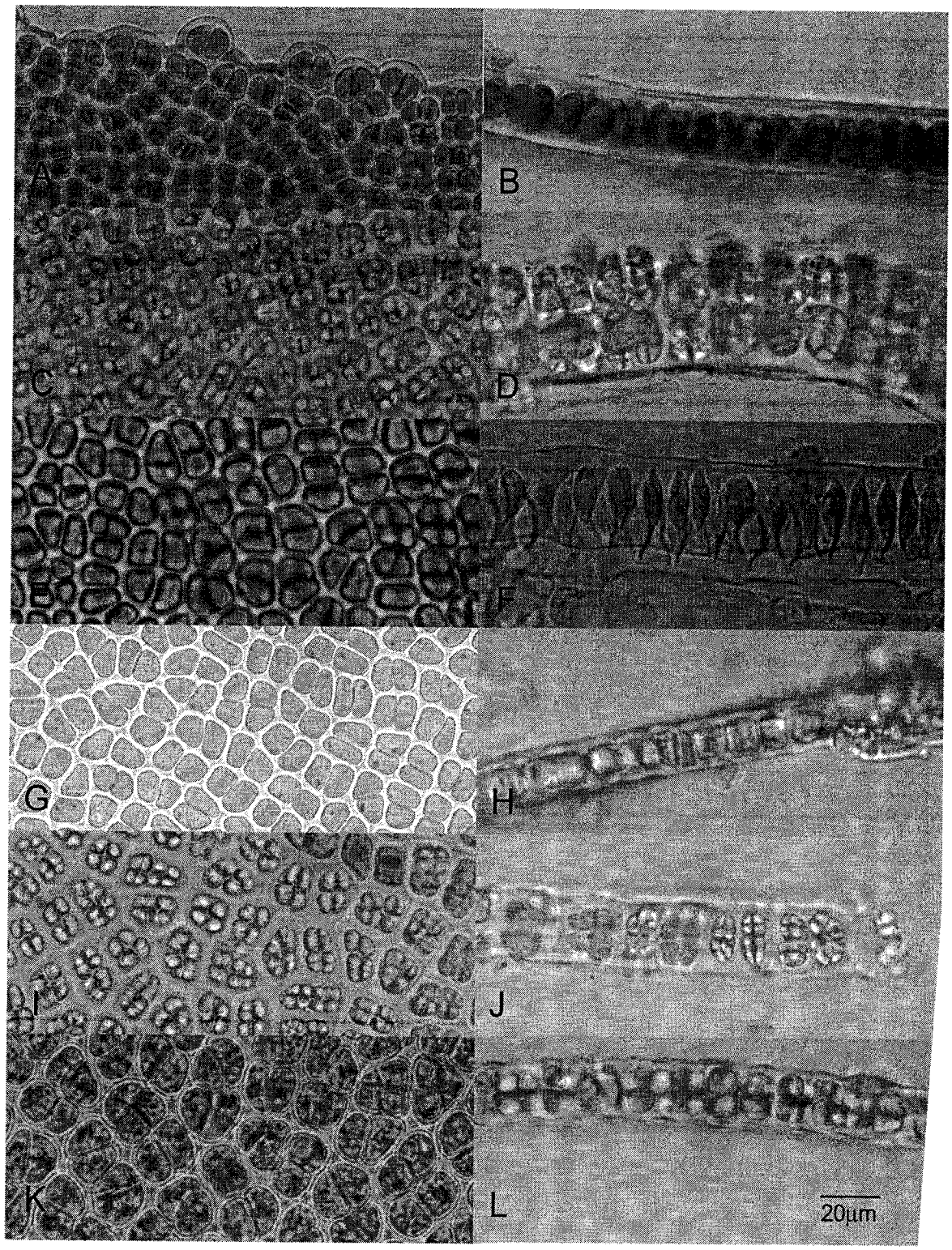


Figure 4.5. Surface and tranverse views of vegetative, male gametangial, and female gametangial thallus regions (A-F) as well as blade morphology of *Porphyra collinsii* NHA78100 (G-I).

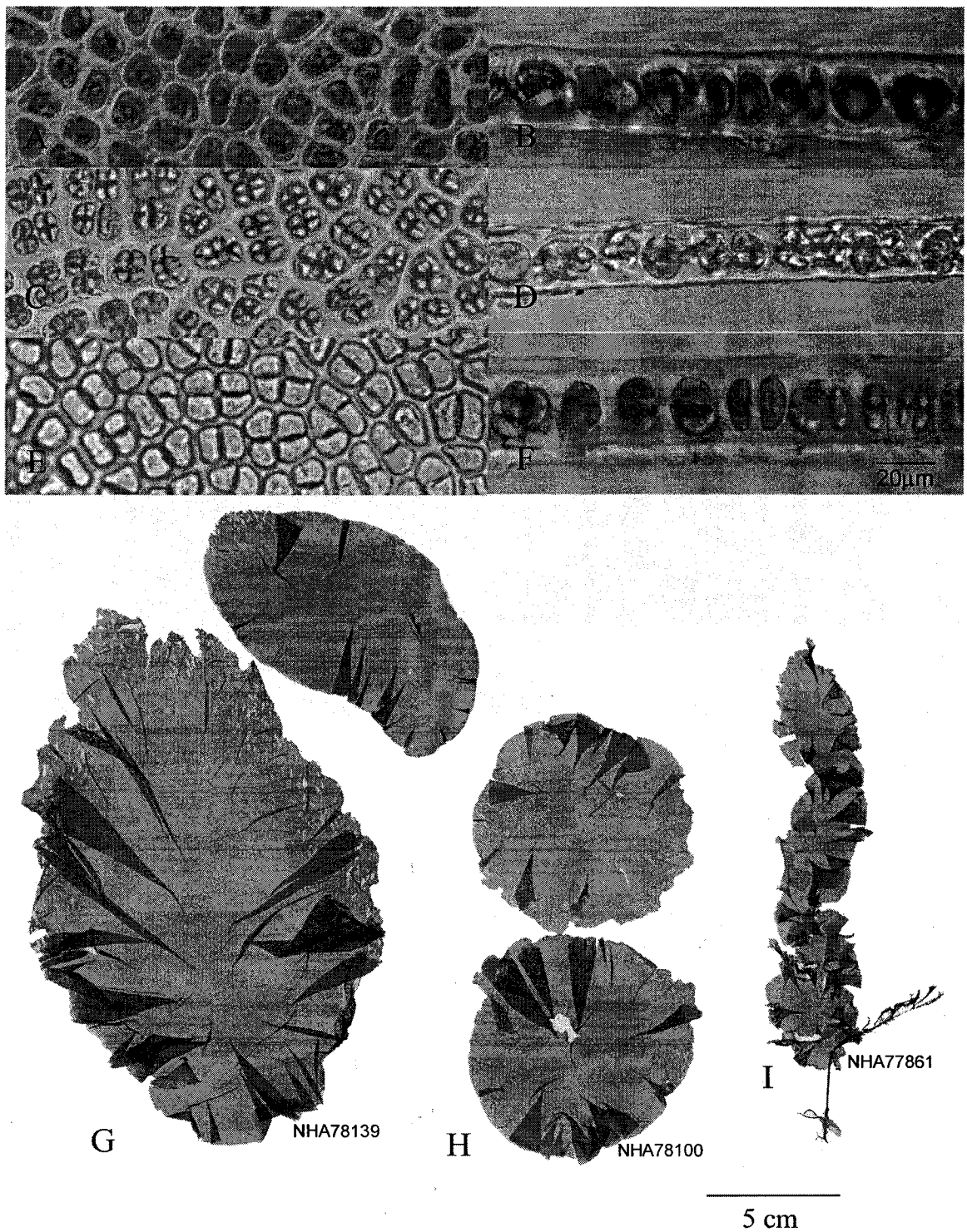
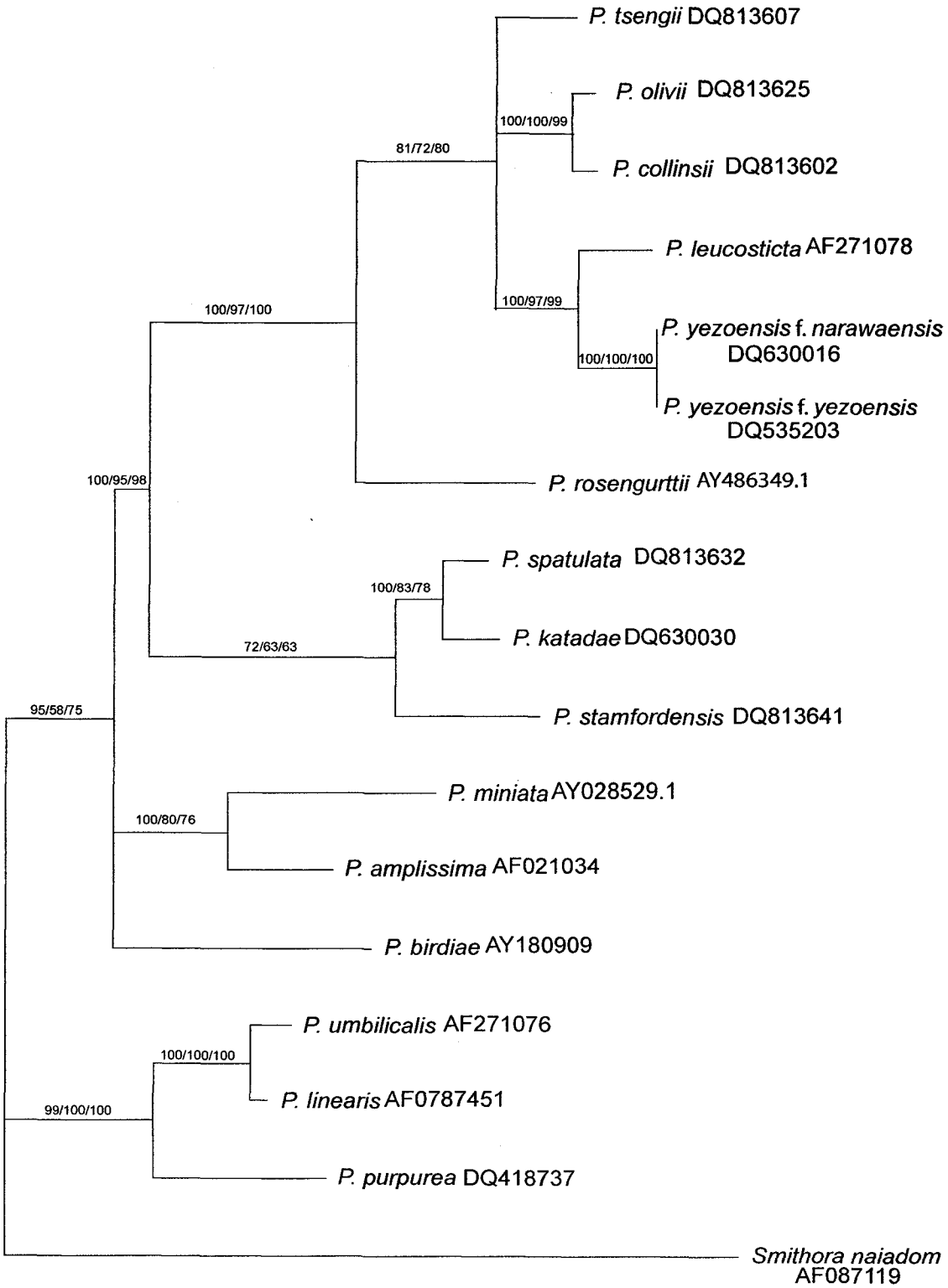


Fig. 6. *rbc*L alignment (1077 bp from position 390 of the *rbc*L to position 27 of the *rbc*L-*rbc*S spacer) of cryptic and known species of Northwest Atlantic *Porphyra*. Support values are presented as Bayesian posterio probabilities, neighbor-joining bootstrap and parsimony bootstrap, respectively.



REFERENCES

Agardh, C. A. (1817): Synopsis algarum scandinaviae. Berlingiana, Lund.

Agard, J. G. (1882): Till algernes systematic. Nija bidrag. Lunds Univ. 'rsskr. **19**:1-182.

Albion, Robert Greenhalgh (1970): The Rise of New York Port. Charles Scribner's Sons. New York.

Allen, F. E. (1953): Distribution of marine invertebrates by ships. Aust. J. Mar. Freshwater Res. **4**:307-316.

Amsler, C. D. & R. B. Searles (1980): Vertical distribution of seaweed spores in a water column offshore of North Carolina. J. Phycol. **16**:617-619.

Anonymous (1941): A Maritime History of New York. Doubleday, Doran and Co. Inc. Garden City, New York.

Apollonio, S. (1979): The Gulf of Maine. Courier of Maine Books, Rockland, Maine

Bird C. & J. McLachlan (1992): Seaweed flora of the Maritimes I. Rhodophyta-The red algae. Biopress, England.

Boney, A. (1965): Aspects of the biology of the seaweeds of economic importance. Adv. Mar. Biol. **3:**105-253.

Børgesen, F. (1902): Marine algae. Botany of the Faeröes. 339-532. *f.* 51-110.

Bray, Troy L., C. D. Neefus & A. C. Mathieson (2006): A morphological and molecular survey of *Porphyra purpurea* (Roth) C. Agardh (Rhodophyta, Bangiales) from the Northwest Atlantic. Nova Hedwigia **82**:1-22.

Breeman, A. M. (1989): Expected effects of changing seawater temperatures on the geographic distribution of seaweed species.-In: Beukema J. J, W. J. Wolff & J. J. W. M. Brouns (eds): Expected effects of climatic change on marine coastal ecosystems: 66-76. Kluwer Academic Publishers, Dordrecht.

Breeman, A. M. & H. Pakker (1994): Temperature ecotypes in seaweeds: adaptive significance and biogeographic implications. Bot. Mar. **37**:171-180.

Briggs, J. C. (1974): Marine zoogeography. McGraw-Hill, New York.

Brodie J., P. K. Hayes, G. L. Barker & L. M. Irvine (1996): Molecular and morphological characters distinguishing two *Porphyra* species (Rhodophyta: Bangiophycidae). Eur. J. Phycol. **31**:303-308.

Brodie J., P. K. Hayes, G. L. Barker, L. M. Irvine & I. Bartsch (1998): A reappraisal of *Porphyra* and *Bangia* (Bangiophycidae, Rhodophyta) in the Northeast Atlantic based on the *rbc*L-*rbc*S intergenic spacer. J. Phycol. **34**:1069-1074.

Brodie J. & Irvine, L. (1997): A comparison of *Porphyra dioica Sp. Nov.* and *P. purpurea* (Roth) C. AG. (Rhodophyta: Bangiophycidae) in Europe. Cryptogamie Algol. **18(3)**; 283-297.

Brodie, J. & L. Irvine (2003): Seaweeds of the British Isles, Vol. 1. Rhodophyta, Pt. 3B. Bangiophycidae, Natural History Museum, London.

Brodie, J, I Bartsch, C. Neefus, S. Orfanidis, T. Bray & A. Mathieson. New insights into the cryptic diversity of the North Atlantic-Mediterranean '*Porphyra leucosticta*' complex: *P. olivii* sp. nov. and *P. rosengurttii* (Bangiales, Rhodophyta). Submitted.

Broom, J. E., W. A. Nelson, D. F. Hill, G. A. Knight & W. A. Nelson (1999): Species recognition in New Zealand *Porphyra* using 18S rDNA sequencing. J. Appl. Phycol. **11**:421-428.

Broom J. E., W. A. Nelson, C. Yarish, W. A. Jones, R. Aguilar Rosas & L. E. Aguilar Rosas (2002): A reassesment of the taxonomic status of *Porphyra orbiculata, Porphyra carolinensis* and *Porphyra lilliputiana* (Bangiales, Rhodophyta) based on molecular and morphological data. Eur. J. Phycol. **37**: 227-236.

Broom, J. E., T. J. Farr, & W. A. Nelson (2004): Phylogeny of the *Bangia* flora of New Zealand suggests a southern origin for *Porphyra* and *Bangia* (Bangiales, Rhodophyta). Mol. Phylogenetics and Evol. **31**:1197-1207.

Bunting, F. (1980): The color shop primer: an introduction to the history of color, color theory, and color measurement. Light Source Computer Images, Inc., An X-Rite Company. Grandville, Michigan.

Campbell, S. (1980): *Palaeoconchocelis starmachii*, a carbonate boring microfossil from the Upper Silurian of Poland (425 million years old): implications for the evolution of the Bangiaceae (Rhodophyta). Phycologia **19**:25-36.

Carlton, J. T. (1987): Patterns of transoceanic marine biological invasions in the Pacific Ocean. Bull. Mar. Sci. **41**:452-465.

Carlton, J. T. (1996): Pattern, process, and prediction in marine invasion ecology. Bio. Conservation **78**:97-106.

Carlton, J. T. & J. B. Geller (1993): Ecological roulette: biological invasions and the global transport of nonindigenous marine organisms. Science **261**:78-82.

Carlton, J. T. & J. Hodder (1995): Biogeography and dispersal of coastal marine organisms: experimental studies on a replica of a 16th-century sailing vessel. Marine Bio. **121**:721-730.

Carlton, J. T. & J. A. Scanlon (1985): Progression and dispersal of an introduced alga: *Codium fragile* ssp. *tomentosoides* (Chlorophyta) on the Atlantic Coast of North America. Bot. Mar. **28**: 155-165.

Carlton, J. T., D. M. Reid, & H. van Leeuwen (1995): The role of shipping in the introduction of nonindigenous aquatic organisms to the coastal waters of the United States (other than the Great Lakes) and an analysis of control options. Washington, DC: U.S. Coast Guard.

Chapman, R. L. (1971): The macroscopic marine algae of the Sapelo Island and other sites on the Georgia coasts. Bull. Georgia Acad. Sci. **29**:77-89.

Clark, D. L. (1982): Origin, nature, and world climate effect of Arctic Ocean ice-cover. Nature **300**:321-325.

Clement M., D. Posada, & K. A. Crandall (2000): TCS: a computer program to estimate gene genealogies. Mol. Ecol. **9**:1657-1659.

Clokie, J. & A. D. Boney (1980): Conchocelis distribution in the Firth of Clyde: estimates of the lower limits of the photic zone. J. Exp. Mar. Biol. Ecol. **46**:111-125.

Coll J. & J. Cox (1977): The genus *Porphyra* C. Ag. (Rhodophtya, Bangiales) in the American North Atlantic. I. New species from North Carolina. Bot. Mar. **20**:155-159.

Collins, F. S. (1882): Notes on New England Algae. Bulletin of the Torrey Botanical Club. **9**(5):69-71.

Collins, F. S. (1900): Preliminary lists of New England plants—V. Marine Algae. Rhodora **2**:41-52.

Collins, F. S. (1908): Notes on Algae—IX. Rhodora **5**:155-164.

Coleman, D. C. & A. C. Mathieson (1974): Investigations of New England marine algae VII: Seasonal occurrence and reproduction of marine algae near Cape Cod, Massachusetts. Rhodora **77**: 76-104.

Coyer, J. A., G. Hoarau, M. Skage, W. T. Stam, & J. L. Olsen (2006): Origin of *Fucus serratus* (Heterokontophyta; Fucaceae) populations in Icelnand and the Faroes: a microsatellite-based assessment. Eur. J. Phycol. **41(2)**:235-246.

Crandall KA (1994): Intraspecific cladogram estimation: accuracy at higher levels of divergence. Syst. Biol **43**:222-235.

Cronk, Q. C. B. (1989): The past and present vegetation of St. Helena. J. Biogeography **16**:47-64.

Curtis, B. A. (1997): A morphological and phylogenetic investigation of *Porphyra linearis* Greville and *Porphyra purpurea* (Roth) C. Agardh forms from Nova Scotia. M.Sc. Thesis. Acadia Univ.

Druehl, L. D. (1981): Geographical distribution. In Lobban S. & M. J. Wynne (eds): The Biology of Seaweeds: 306-325. Blackwell Scientific Publications, Oxford, England.

Emiliani, C. (1961): The temperature decrease of surface sea-water in high latitudes and of abyssal-hadal water in open oceanic basins during the past 75 million years. Deep-Sea Res. **8**:144-147.

Fallaw, W. C. (1979): Trans-North Atlantic similarity among Mesozoic and Cenozoic invertebrates correlated with widening of the ocean basin. Geology **7**:398-400.

Fallow, W. C. & Dromgoole, El. L. (1980): Faunal similarities across the South Atlantic among Mesozoic and Cenozoic invertebrates correlated with widening of the ocean basin. J. Science **283**:166-72.

FAO 2003. Review of the State of World Aquaculture. FAO Fisheries Circular No. 886, Rev. 2. pp 95. Electronic edition: http://www.fao.org/DOCREP/005/Y4490E/Y4490E00.HTM

Farris, J. S. (1989): The retention index and the rescaled consistency index. Cladistics **5**:417-419.

Farr, T. J., W. A. Nelson & J.E.S. Broom (2003): A challenge to the taxonomy of *Porphyra* in Australia: the New Zealand red alga *Porphyra rakiura* (Bangiales, Rhodophyta) occurs in southern Australia, and is distinct from *P. lucasii.* Aust. Syst. Botany **16**:569-575.

Fofonoff, P. W., G. M. Ruiz, B. Steves, & J. T. Carlton. In Ships or on Ships? (2003): Mechanisms of Transfer and Invastion for Nonnative Species to the Coasts of North America -In: Ruiz G. M. & J. T. Carlton (eds) Invasive Species: Vectors and Management Strategies: 52-182. Island Press, Washington.

Franz, D. R. & A. S. Merrill (1980): The origins and determinants of distribution of molluscan faunal groups on the shallow continental shelf of the northwest Atlantic. Malacologia **19**:227-248.

Germano J. & A. S. Klein (1999): Species-specific nuclear and chloroplast single nucleotide polymorphisms to distinguish *Picea glauca, P. mariana* and *P. rubens.* Theor. Appl. Genet. **99**: 37-49.

Getchis, T. (2005): An assessment of the needs of Connecticut's shellfish aquaculture industry. CTSG-05-02. Connecticut Sea Grant College Program. Groton, CT. 12pp.

Gollash, S. (1996): *Untersuchungen des Arteintrages durch den internacionales Schiffsverskehr unter besonderer Berücksichtigung nichtheimischer Arten.* PhD Thesis, Dr. Kovac, Hamburg.

Goodenough, S & T. J. Woodward (1797): Observations on the British Fuci, with particular descriptions of each species. Transactions of the Linnean Society of London. **3**:84-235, Plates 16-19.

Greville, R. K. (1830): *Algae britannicae . . .* Baldwin and Craddock, Endinburgh. lxxxviii + 218 pp.

Griffin, N. J., J. J. Bolton, & R. J. Anderson (1999): Distribution and population dynamics of *Porphyra* (Bangiales, Rhodophyta) in the southern Western Cape, South Africa. J. Appl. Phyco. **11**:429-436.

Guiry M. D. (1990): Sporangia and spores. In: Cole, K. M. & R. G. Sheath (eds.), Biology of the Red Algae, Cambridge Univ. Press., New York and Cambridge, pp. 347-376.

Hallegraeff, G. M. & C. J. Bolch (1991): Transport of toxic dinoflagellates cysts via ships' ballast water. Mar. Pollution Bulletin **22**:27-30.

Hall, A. (1981): Copper accumulation in copper-tolerant and non-tolerant populations of the marine fouling alga *Ectocarpus siliculosus* (Dillw.) Lyngbye. Bot. Mar. **24**:223-228.

Hallam, A. (1981): Relative importance of plate movements, eustasy, and climate in controlling major biogeographical changes since the Early Mesozoic.-In Nelson

G. & De. E. Rosen (eds): Vicariance biogeography: 303-340. Columbia University Press, NewYork.

Hallam, A. (1994): An Outline of Phanerozoic Biogeography. -Oxford University Press, Oxford, England.

Harrison, P. & R. Bigley (1982): The recent introduction of the seagrass *Zoster japonica* Aschers and Graebn to the Pacific coast of North America. Can. J. Fish Aqua. Sc. **39**:1642-1648.

Hay, M. E. & S. D. Gaines (1984): Geographic differences in herbivore impact: do Pacific herbivores prevent Caribbean seaweeds from colonizing via the Panama Canal? Biotropica **16**:24-30.

Harvey, W. E. (1858): Nereis Boreali-Americana. Part I. Melanospermeae. 1-218 *pl.*1-12.

Herman, Y. & D. M. Hopkins (1980): Arctic oceanic climate in late Cenozoic time. Science **209**:557-562.

Hickey, L. J., R. M. West, M. R. Dawson, & D. K. Choi (1983): Arctic terrestrial biota: paleomagnetic evidence of age disparity with mid-northern latitudes during the Late Cretaceous and early Tertiary. Science **221**:1153-1156.

Hines, A. H., A W. Miller, G. M. Ruiz, & K. Lion (2004): Estimating domestic and foreign ballast water as a vector for invasive species: regional analysis for New England, Northeast North America. –In: Pederson (ed): Ballast water exchange: exploring the feasibility of alternative ballast water exchange zones in the North Atlantic. Massachusetts Institute of Technology, Cambridge.

Hoek, C. van den. (1975): Phytogeographic provinces along the coasts of the northern Atlantic Ocean. Phycologia **14**:317-330.

Hoek, C. van den. (1984): World-wide latitudinal and longitudinal seaweed distribution patterns and their possible coauses, as illustrated by the distribution of Rhodophytan genera. Helgoländer Meeresunters **38**:227-257.

Hoek, C. van den. (1987): The possible significance of long-range dispersal for the biogeography of seaweeds. Helgoländer Meeresunters **41**:261-272.

Hoek, C. van den & M. Donze (1967): Algal phytogeography of the European Atlantic coasts. Blumea; tijdschrift voor de systematiek en de geografie der planten; a journal of plant taxonomy and plant geography **15(1)**:63-89.

Hollenberg, G. (1958): Culture studies of marine algae. III. *Porphyra perforata.* Amer. J. of Botany. **45**(9):653-656.

Holmes, M. J. & J. Brodie (2004): Morphology, seasonal phenology and observations on some aspects of the life history in culture of *Porphyra dioica* (Bangiales, Rhodophyta) from Devon, UK. Phycologia. **43**:176-188.

Hopkins, D. M. (1967): The Bering land bridge. -Stanford University Press, Stanford, Calif.

Howe, Christopher (1996): The Origins of Japanese Trade Supremacy. The University of Chicago Press, Chicago.

Howe, M. A. (1911): Phycological studies-V. Some marine algae of lower California, Mexico. Bulletin of Torrey Botanical Club. **38**(11):489-514.

Hruby, T. & T. A. Norton (1979): Algal colonization on rocky shores in the Firth of Clyde. J. Eco. **67**:65-77.

http://www.maine.gov/dmr/aquaculture/leaseinventory2005/newmeadowsriver.htm. Aquaculture Lease Inventory - New Meadows River.

http://www.mass.gov/czm/wpshell.htm. Massachusetts Aquaculture White Paper. Shellfish bottom and off-bottom culture.

http://www.sis.gov.eg/calendar/html/c1171196.htm. The Inaugration of the Suez Canal.

Hus, H. T. (1900): Proc. California Acad. Sci. III. 2:202. Zoe **5**:63.

Hus, H. T. A. (1902): An account of the species of *Porphyra* found on the Pacific coast of North America. Proc. Calif. Acad. Sc., 3rd Ser. (Bot.) **2**:173-236.

Hutchings, P. A., R. W. Hillard, & S. L. Coles (2002): Species introductions and potential for marine pest invasions into tropical marine communities, with special reference to the Indo-Pacific. Pac. Sc. **56(2)**:223-233.

Ingolfsson, A. (1992): The Origin of the Rocky shore Fauna of Iceland and the Canadian Maritimes. J. Biogeography **19(6)**:705-712.

Ishikawa, M. (1921): Cytological studies on *Porphyra tenera* Kjellm. I., Bot. Mag. Tokyo **35**(419): 206-218.

Jensen A. (1993): Present and future needs for algae and algal products. Hydrobiologia **260/261**:15-23.

John, D. M. (1974): New records of *Ascophyllum nodosum* (L.) Le Jol. From the warmer parts of the Atlantic Ocean. J. Phycol. **10**:243-244.

Jokiel, P. L. (1984): Long distance dispersal of reef corals by rafting. Coral Reefs **3**:113-116.

Jones, W. A., N. J. Griffin, D. T. Jones, W. A. Nelson, T. J. Farr, & J. E. Broom (2004): Phylogenetic diversity in South African *Porphyra* (Bangiales, Rhodophyta) determined by nuclear SSU sequence analyses. Eur. J. Phycol. **39**:197-211.

Jönsson, H. (1912): The marine algal vegetation. -In Warming, E. & L. K. Rosevinge (eds): The Botany of Iceland: 1:1-186. *f.* 1-7.

Jónsson, S. (1970): Meeresalgen als Erstbesiedler der Vulkaninsel Surtsey. – Schr. Naturw. Ver. Schlesw.-Holst. (Sonderb.: Surtsey Isand, natürliche Erstbesiedlung [Ökogenese] der Vulkan-insel): 21-28.

Kapraun D. F., T. K. Hinson & A. J. Lemus (1991): Karyology and cytophotometric estimation of interspecific and intraspecific nuclear DNA variation in four species of *Porphyra* (Rhodophyta). Phycologia **30:**458-466.

Kennett, J. P. (1982): Marine Geology. -Prentice-Hall, Englewood, New Jersey.

Kjellman, F. R. (1883): The Algae of the Arctic Sea. -*Kongl. Boktryckeriet,* Stockholm.

Klein, A. S., A. C. Mathieson, C. D. Neefus, D. F. Cain, H. A. Taylor, B. W. Teasdale, A.L. West, E. J. Hehre, J. Brodie, C. Yarish, C. & A. L. Wallace (2003): Identification of north-western Atlantic *Porphyra* (Bangiaceae, Bangiales) based on sequence variation in nuclear SSU and plastid *rbc*L genes. Phycologia **42**:109-122.

Kluge, A. G. & J. S. Farris (1989): Quantitative phyletics and the evolution of anurans. Syst. Zool. **18**:1-32.

Knott, S. T. & H. Hoskins (1968): Evidence of Pleistocene Events in the Structure of the Continental Shelf off the Northeastern United States. Marine Geology **6**:5-43.

Kornman, P. (1986): *Porphyra yezoensis* bei Helgoland- eine entwichlungsgeschichtliche Studie. Helgol. Wiss. Meeresunters. **40**:327-342

Kornman, P. (1994): Life histories of monostromatic *Porphyra* species as a basis for taxonomy and classification. Eur. J. Phycol. **29**:69-71.

Kornman, P. & P. H. Sahling (1991): The *Porphyra* species of Helgoland (Bangiales, Rhodophyta). Helgol Wiss Meeresunters **45**:1-38.

Kunimoto, M.., H. Kito, Y. Kaminishi & N. Murase (1999a): Molecular divergence of the SSU rRNA gene and internal transcribed spacer 1 in *Porphyra yezoensis* (Rhodophyta). J. Appl. Phycol. **11**: 211-216.

Kunimoto, M., H. Kito, Y. Mizukami, N. Murase, & I. Levine (1999b): Molecular features of a defined genetic marker for the determination of the *Porphyra tenera* lineage. J. Appl. Phycol. **15**:337-343.

Kunimoto, M., H. Kito, Y. Mizukami, N. Murase, & I. Levine (2003): Molecular features of a defined genetic marker for the determination of the *Porphyra tenera* lineage. J. Appl. Phycol. **15**:337-343.

Kützing, F. T. (1843): Phycologia generalis oder Anatomie Physiologie und Systematik der Tange. Leipzig.

Lamouroux, J. V. (1825): Gélidie *Dictionnaire Classique d'Histoire Naturelle.* **7**:190-191.

Lawver L. A., R. D. Miller, S. P. Srivastava, & W. Roest (1990): The opening of the Arctic Ocean. –In: Bleil, U & J. Thiede (eds): Geological history of the polar oceans: Arctic vs Antarctic: 29-62. Kluwer Academic Publishers, The Netherlands.

LeJolis, A. (1863): Liste des algues marines de Cherbourg. *Mémoires de la Société Impériale des Sciences Naturelles de Cherbourg* 10:5-168, VI pls., VI plates.

Levine I., (1998): Commercial cultivation of *Porphyra* (nori) in the United States World Aquaculture **29**:37-47.

Lindstrom, S. C. (1987): Possible sister groups and phylogenetic relationships among selected North Pacific and North Atlantic Rhodophyta. Helgoländer Meeresunters. **41**:245-260.

Lindstrom, S. C. (2001): The Bering Strait connection: dispersal and speciation in Boreal macroalgae. J. Biogeography **28**:243-251.

Lindstrom, S. C. & K. M. Cole (1992a): Relationships between some North Atlantic and North Pacific species of *Porphyra* (Bangiales, Rhodophyta): evidence from isozymes, morphology, and chromosomes. Can. J. Bot. **70**:1355-1362.

Lindstrom, S. C. & K. M. Cole (1992b): A revision of the species of *Porphyra* (Rhodophyta: Bangiales) occurring in British Columbia and adjacent waters. Can. J. Bot. **70**: 2066-2075.

Lindstrom, S. C. & Cole, K. M. 1993. The systematics of *Porphyra*: character evolution in closely related species. Hydrobiologia **260/261**:151-157.

Lindstrom, S. C. & S. Fredericq (2003): *rbc*L gene sequences reveal relationships among north-east Pacific species of *Porphyra* (Bangiales, Rhodophyta) and new species *P. aestivalis.* Phycol. Res. **51**:211-224.

Lubchencho, J. & J. Cubit (1980): Heteromorphic life histories of certain marine algae as adaptations to variations in herbivory. Ecology **61** (3):676-687.

Lüning, Klaus (1990): Seaweeds. Their Environments, Biogeography, and Ecophysiology. -John Wiley & Sons, Inc. New York.

Lyngbye, H. C. (1819): *Tentamen hydrophytologiae danicae. . .* Shultz, Copenhagen, xxxii-248p.

MacGaughey, V. (1918): Algae of the Hawaiian archipelago. II. Botanical Gazette **65**(2):121-149.

Mack, R. N. (2003): Global Plant Dispersal, Naturalization, and Invasion: Pathways, Modes, and Circumstances. –In: Ruiz G. M. & J. T. Carlton (eds): Invasive Species: Vectors and Management Strategies: 3-30. Island Press, Washington.

Malinowski, K. & J. Ramus. (1973): Growth of the green algal *Codium fragile* in a Connecticut estuary. J. Phycol. **9**:102-110.

Malinowski, K. C. (1974): *Codium fragile*- the ecology and population biology of a colonizing species. Ph. D. dissertation, Yale Univ., New Haven, Connecticut.

Marincovich, L. E. M. Brouwers Jr., D. M. Hopkins, & M. C. McKenna (1990): Late Mesozoic and Cenozoic paleogeographic history of the Arctic Ocean basin, based on shallow-water marine faunas and terrestrial vertebrates. –In: Grantz A., L. Johnson and F. Sweeney (eds): The Arctic Ocean region: the geology of North America. Vol. I: 403-426. Geological Society of America, Boulder Colorado.

Martindale, I. C. (1877): More about ballast plants. U.S. Botanical Gazette **2**:127-128.

Mathieson, A. C. (1979): Vertical distribution and longevity of subtidal seaweeds in northern New England. Bot. Mar. **22**: 511-520.

Mathieson, A. C., C. J. Dawes, & E. J. Hehre (1998): Floristic and zonation studies of seaweeds frm Mount Desert Island, Maine: an historical comparison. Rhodora **100**: 333-379.

Mathieson, A. C., C. J. Dawes, M. L. Anderson, & E. J. Hehre (2001): Seaweeds of the Brave Boat Harbor salt marsh and adjacent open coast of southern Maine. Rhodora **103**: 1-46.

Mathieson, A. C., C. J. Dawes, L.G. Harris & E. J. Hehre (2003): Expansion of the Asiatic green alga *Codiuim fragile* subsp. *tomentosoides* in the Gulf of Maine. Rhodora **105**: 1-53.

Mathieson, A. C. & R. A. Fralick (1972): Investigations of New England marine algae. V. The algal vegetation of the Hampton-Seabrook Estuary and the open coast near Hampton, New Hampshire. Rhodora **74**: 406-435.

Mathieson, A. C. & E. J. Hehre (1986): A synopsis of New Hampshire seaweeds. Rhodora **88**:1-139.

Mathieson, A. C., E. J. Hehre, & M. Costa (1993): Algal vegetation of the York River Estuary and the adjacent open coast of southern Maine. Rhodora **95**: 285-324.

Mathieson, A. C., E. J. Hehre, J. Hambrook & J. Gerweck (1996): A comparison of insular seaweed floras from Penobscot Bay, Maine and other northwest Atlantic islands. Rhodora **98**: 369-418.

Mathieson, A. C., C. D. Neefus, & C. Emerich-Penniman (1983): Benthic ecology in an estuarine tidal rapid. Bot. Mar. **26**: 213-230.

Mathieson, A. C. & C. A. Penniman (1986a): Species composition and seasonality of New England seaweeds along an open coastal-estuarine gradient. Bot. Mar. **29**: 161-176.

Mathieson, A. C. & C. A. Penniman (1986b): A phytogeographic interpretation of the marine flora from the Isles of Shoals, U.S.A. Bot. Mar. **29**: 413-434.

Mathieson, A. C. & C. A. Penniman (1991): Floristic patterns and numerical classification of New England estuarine and open coastal seaweed populations. Nova Hedwigia **52**: 453-485.

Mathieson, A. C., N. B. Reynolds, & E. J. Hehre (1981): Investigations of New England marine algae II: The species composition, distribution and zonation of seaweeds in the Great Bay Estuary System and the adjacent open coast of New Hampshire. Bot. Mar. **24**: 533-545.

McHugh, D. J. (2003): A guide to the seaweed industry. FAO Fisheries Technical Paper, 441.

McIntyre, A. (1976): The surface of the ice-age earth. Science **191**:1131-1137.

Michanek, G. (1979). Phytogeographic provinces and seaweed distribution. Bot. Mar. **22**:375-391.

Mills, E. L., J. H. Leach, J. T. Carlton, & C. L. Secor (1993): Exotic species in the Great Lakes: a history of biotic crises and anthropogenic introductions. J. Great Lakes Res. **19**:1-54.

Mitman G. G. & J. P. van der Meer (1994): Meiosis, blade development, and sex determination in *Porphyra purpurea* (Rhodophyta). J. Phycol. **30**: 147-159.

Miura A. (1968): *Porphyra katadae*, a new species from Japanese coast. J. Tokyo Univ. Fish. **54**: 55-59.

Miura A. (1984): A new variety and a new form of *Porphyra* (Bangiales, Rhodophyta) from Japan: *Porphyra tenera* Kjellman var. *tamatsuensis* Miura var. nov. and *P. yezoensis* Ueda form. *narawaensis* Miura form. nov. J. Tokyo Univ. Fish. **71**: 1-14.

Miura A. (1988): Taxonomic studies of *Porphyra* species cultivated in Japan, referring to their transition to the cultivated variety. J. Tokyo Univ. Fish. **75**: 311-325.

Miura, A. & Y. Aruga (1987): Distribution of *Porphyra* in Japan as affected by cultivation. J. Tokyo Univ. Fish. **74(1)**:41-50.

Mizukami, Y., H. Kito, Y. Kaminishi, N. Murase, & M. Kunimoto (1999): Nucleotide sequence variation in the ribosomal internal transcribed spacer regions of cultivated (cultivars) and field-collected thalli of *Porphyra yezoensis.* Fisheries Science **65(5)**:788-789.

Morse, S. R. & F. S. Collins (1888): Algae from Atlantic City, N. J. Bull. Torrey Bot. Club **15(12)**:309-314.

Moss, B. L., D. Tovey, & P. Court (1981): Kelps as fouling organisms on North Sea platforms. Bot. Mar. **24**:207-209.

Müller K. M., J. J. Cannone, R. R. Gutell, & R. G. Sheath (2001): A structural and phylogenetic analysis of the group ICI introns in the order Bangiales (Rhodophyta). Molecular Biology and Evolution **18**:1654-1667.

Müller, K., R. Sheath, V. Morgan, T. Crease, and K. Cole (1998): Biogeography and systematics of *Bangia* (Bangiales, Rhodophyta) based on the rubisco spacer, *rbc*L gene and 18S rRNA gene sequences and morphometric analysis. 1. North America. Phycologia **37(3)**:195-207.

Mumford T. F. & A. Miura (1988): *Porphyra* as a food: cultivation and economics. -In: Lembi C. A. & J. R. Waaland (eds): Algae and Human Affairs: 87-117. Cambridge University Press, London.

National Research council (1996): Stemming the tide: controlling introductions of nonindigenous species by ships' ballast water. -National Academy Press, Washington.

Neefus, C. D., A. C. Mathieson, T. L. Bray, & C. Yarish: The occurrence of three introduced Asiatic species of *Porphyra* (Bangiales, Rhodophyta) in the northwestern Atlantic. Submitted.

Neefus, C. D., A. C. Mathieson, C. Yarish, A. S. Klein, A. West, B. W. Teasdale & E. J. Hehre (2000): Five cryptic species of *Porphyra* from the Northwest Atlantic. J. Phycol. **36**: 3 (supplement).

Neefus, C. D., A. C. Mathieson, A. S. Klein, B. Teasdale, T. Bray, & C. Yarish (2002): *Porphyra birdiae sp. nov.* (Bangiales, Rhodophyta): a new species from the Northwest Atlantic. Algae **17(4)**: 203-16.

Nei, M. (1987): Molecular Evolutionary Genetics. -Columbia University Press, New York, New York.

Nelson, W. A., J. Brodie & M. D. Guiry (1999): Terminology used to describe reproduction and life history stages in the genus *Porphyra* (Bangiales, Rhodophyta). J. Appl. Phycol. **11**: 407-410.

Nelson, W. A., J.E. Broom, & T. J. Farr (2003): *Pyrophyllon* and *Chlidophyllon* (Erythropeltidales, Rhodophyta): two new genera for obligate epiphytic species previously placed in *Porphyra*, and a discussion of the orders Erythropeltidales and Bangiales. Phycologia **42**:308-315.

Nelson, W. A., T. J. Farr, & J.E.S. Broom (2006): Phylogenetic relationships and generic concepts in the red order Bangiales: challenges ahead. Phycologia **45**(3): 249-259.

Niwa, K. & Y. Aruga (2003): Rapid DNA extractions from conchocelis and ITS-1 rDNA sequences of seven strains of cultivated *Porphyra yezoensis* (Bangiales, Rhodophyta). J. Appl. Phycol. **15**:29-35.

Niwa, K., N. Kikuchi, M. Iwabuchi & Y. Aruga (2004): Morphological and AFLP variation of *Porphyra yezoensis* Udea form. *narawaensis* Miura (Bangiales, Rhodophyta). Phycol. Res. **52**: 180-190.

Niwa, K., N. Kikuchi, & Y. Aruga (2005): Morphological and molecular analysis of the endangered species *Porphyra tenera* (Bangiales, Rhodophyta). J. Phycol. **41**:294-304.

Noda, H., Y. Yoriguchii, & S. Araki (1975): Studies on the flavor substances of "nori," the dried laver *Porphyra spp.*-II. Free amino acids and 5' nucleotides. Bull. Jap. Soc. Sci. Fish. **41**:1299-1303.

Norton, T. A. & A. C. Mathieson (1983): The biology of unattached seaweeds. Progr. Phycol. Res. **2**:333-386.

Olivera, M.C., J. Kurniawan, C. J. Bird, E. L. Rice, C. A. Murphy, R. K. Singh, R. R. Gutell, & R. A. Ragan (1995): A preliminary investigation of the order Bangiales (Bangiophycidiae, Rhodophyta) based on sequences of the nuclear small-subunit ribosomal RNA genes. Phycol. Res. **43**:71-79.

Perestenko L.P. (1994): Red Algae of the far-eastern seas of Russia. -Komarov Botanical Institute, Russian Academy of Sciences, St. Petersburg.

Pielou, E. C. (1977): The latitudinal spans of seaweed species and their patterns of overlap. J.Biogeography **4**(1):299-311.

Pitman, W. C. & M. Talwani (1972): Sea-floor spreading in the North Atlantic. Bull. Geol. Soc. Am. **83**:619-646.

Por, F. D. (1978): Lessepsian Migration: the influx of Red Sea Bota into the Mediterranean. -Springer; Berlin

Por, F. D. (1990): *Lessepsian* migrations. An appraisal and new data. Bull. Inst. Océanographique, Monaco **7**:1-10.

Porter, A. N., ed. (1991): Atlas of British Overseas Expansions. –Routledge, London.

Ragan, M. A., C. J. Bird, E. L. Rice, R. R. Gutell, C. A. Murphy, & R. K. Singh (1994): A molecular phylogeny of the marine red algae (Rhodophyta) based on the nuclear small subunit rRNA gene. Proc. Nat. Acad. Sci. U.S.A. **91**:7276-80.

Reinke, J. (1889): Algenflora der westlichen Ostsee deutschen Antheils. Ber. D. Komm. Z. wiss. Unters. D. deut. Meere in Kiel. 6:III-XI and 1-101. *f.* 1-8. 1 *col. map.*

Reynolds, N. B. (1971): The ecology of a New Hampshire estuarine tidal rapid. PhD. dissertation, University of New Hampshire, Durham, NH.

Rhoads, A. F. & W. M. Klein (1993): The Vascular Flora of Pennsylvania: Annotated Checklist and Atlas. American Philosophical Society, Philadelphia.

Ribera, M. & C. Boudouresque (1995): Introduced marine plants, with special reference to macroalgae: mechanisms and impact. –In: F. E. Round F. E. & D. J. Champman (eds): Progress in Phycological Research, Vol. 11: 187-268, Biopress Ltd., Bristol, England.

Riggs, S. R., S. W. Snyder, A. C. Hine, & D. L. Mearns (1996): Hardbottom Morphology and Relationship to the Geological Framework: Mid Atlantic Continental Shelf. J. Sedimentary Res. **66**(4):830-846.

Roos P. J. (1979): Two stage life cycle of a *Cordylophora* population in the Netherlands. Hydrobiologia **62**:231-239.

Roth, A. G. 1797. Catalecta botanica. I. G. Mülleriana, Leipzig.

Ruiz, G. M., A. W. Miller, K. Lion, B. Steves, A. Arnwine, E. Collinetti, & E. Wells (2001): Status and trends of ballast water management in the United States. First biennial report of the National Ballast Information Clearinghouse. Submitted to U.S. coast Guard.

Ruiz, G. M., A. W. Millier, B. Steves, & R. A. Everett (2003): Global shipping patterns and marine bioinvasions: the hull story? In review.

Saga, N. & Y. Kitade (2002): *Porphyra*: A model plant in marine sciences. Fish. Science **68**:1075-1078.

Saunders, D. A. (1901): Papers from the Harriman Alaskan Expedition. XXV. The Algae. Wash. Acad. Sci., Proc. **3**:391-486.

Saunders, G. W. & G. T. Kraft. (1994): Small-subunit rRNA gene sequences from representatives of selected families of the Gigartinales and Rhodomeniales (Rhodophyta). 1. Evidence for the Plocamiales ord. nov. Can. J. Bot. **72**:1250-1263.

Schneider C. W. & R. B. Searles (1991): Seaweeds of the Southeastern United States. Duke University Press, Durham and London.

Schroeder, B. (1912): Zellpflanzen Ostafrikas gesammelt sur der akademischen Studienfahrt. Hedwigia **52**:288-315.

Sears, J. R. & R. T. Wilce (1975): Sublittoral, benthic marine algae of southern Cape Cod and adjacent Island: seasonal periodicity, associations, diversity, and floristic composition. Ecol. Mono. **45**(4):337-365.

Searles, R. B. (1984): Seaweed biogeography of the mid-Atlantic coast of the United States. Helgoländer Meeresunters **38**:259-271.

Setchell, W. A. (1915): The Law of Temperature connected with the distribution of the marine algae. Annals of the Missouri Botanical Garden. **2**:287-305.

Setchell, W. A. (1917): Geographical Distribution of the Marine Algae. Science. **45**:197-204.

Setchell W. A. & Gardner, N. L. (1903): Algae of northwestern America. Univ. Cal. Publ., Bot. **1**:165-418. *pl. 17-27.*

Siguan, M. A. R. (2003): Pathways of Biological Invasions of Marine Plants. –In: Ruiz G. M. & J. T. Carlton (eds): Invasive Species: Vectors and Management Strategies: 183-226. Island Press, Washington.

Silva P. C. *Porphyra from the Index Nominum Algarum.* http: // ucjeps.berkeley.edu/rlmoe/porphyra.html. Univ. Herbarium, UC Berkley. Rev: 12/20/99.

Simmons, H. G. (1906): Remarks about the relations of the floras of the Northern Atlantic, the Polar Sea, and the Northern Pacific. Bot. Zl. **19**:149-194.

Skottsberg, K. (1906): Observations on the vegetation of the Antarctic Sea. Botany Studies 245-264. *pl.* 7-9. *1 map.*

Smith, J. (1999): Australian Driftseeds: A Compendium of Seeds and Fruits Commonly Found on Australian Beaches. -Armidale: University of New England.

South, G. R. (1983): Benthic marine algae. Biogeography and ecology of the island of Newfoundland. Monographiae Biologicae (The Hague) **48:**385-420.

Srivastava, S. (1985): Evolution of the Eurasian basin and its implication to the motion of Greenland along Nares Strait. Tectonophysic **114**:29-53.

Steane, D. A., B. A. McClure, A. E. Clarke & G. T. Kraft (1991): Amplification of the polymorphic 5.8S rRNA gene from selected Australian Gigartinalean species (Rhodophyta) by polymerase chain reaction. J. Phycol. **27**: 758-762.

Stiller, J. W. & J. R. Waaland (1993): Molecular analysis reveals cryptic diversity in *Porphyra* (Rhodophyta). J. Phycol. **29**: 506-517.

Stiller, J. W. & J. R. Waaland (1996): *Porphyra rediviva sp. nov.* (Rhodophyta): A new species from the northeast Pacific salt marshes. J. Phycol. **32**:323-332.

Suto S. (1950): Studies on shedding, swimming, and fixing of spores of seaweeds. Bull. Jap. Soc. Sci. Fish. **16**:1-9.

Suto, S. (1972): Variation in species characters of *Porphyra* under culture conditions. –In: Abbott I. A & M. Kurogi, (eds): Contributions to the Systematics of Benthic Marine Algae of the North Pacific: 193-201. Japanese Society of Phycology, Kobe, Japan.

Swofford, D. L. (1998): PAUP*; *Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.* Sinauer Associates, Sunderland, MA.

Tappan, H. (1976): Possible eukaryotic algae (Bangiophycidae) among early Proterozoic microfossils. Geo. Soc. America Bull. **87**:633-639.

Taylor, W. R. (1957): Marine algae of the northeastern coast of North America. -Univ. of Michigan Press, Ann Arbor.

Teasdale, B. W. (2004): An investigation of genetic variation within Northwest Atlantic *Porphyra* (Bangiales, Rhodophyta) with specific phylogeographic analysis of the common rocky intertidal species, *Porphyra umbilicalis*. PhD dissertation. University of New Hampshire, Durham, NH.

Teasdale, B., A. West, H. Taylor & A. Klein (2002): A simple restriction fragment length polymorphism (RFLP) assay to discriminate common *Porphyra* (Bangiophyceae, Rhodophyta) taxa from the Northwest Atlantic. J. Appl. Phycol. **14**: 293-298.

Templeton A. R., K. A. Crandall & C. F. Sing (1992): A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**:619-633.

Thompson, J. D. (1991): The biology of a successful invader: what makes *Spartina anglica* so good? BioScience **41**:393-401.

Tseng C.K. (1984): Common Seaweeds of China. Beijing: -Science Press, Beijing.

Tsujii, K., T. Ichikawa, Y. Nakagawa, Y. Matusuura, & M. Kawamura (1983): Hypercholesterolemic effect of taurocyamine or taurine on the cholesterol metabolism in white rats. Sulfur Amino Acids **6**:239-248.

Turner, D. (1802): *A synopsis of the British Fuci.* Pp. xlvi + 400 Yarmouth: -Privately Printed by F. Bush.

Turner, N. J. (2003): The ethonobotany of edible seaweed (*Porphyra abbottae* and related species; Rhodophyta: Bangiales) and its use by First Nations on the Pacific coast of Canada. Can. J. Bot.. **81**:283-293.

Ueda, S. (1932): Nippon san amanori zoku no bunruigaku-teki kenkyu (A systematic study of genus *Porphyra* found on the Japanese coast) J. Imp. Fish. Inst. **28**(1): 1-45, pl. I-XXIV.

Van Andel, T. H. (1985): New Views on an Old Planet. -Cambridge University Press. New York.

Van Oppen M. J. H., S. G.. A. Draisma. L. Olsen, & W. T, Stam (1995): Multiple trans-Arctic passages in the red alga *Phycodrys rubens*: evidence from nuclear rDNA ITS sequences. Mar. Biol. **123**:179-188.

Valentine, J. W. (1973): Evolutionary paleoecology of the marine biosphere. -Prentice Hall, Englewood Cliffs, New Jersey.

Verlaque, M. (2001): Checklist of the macroalgae of Thau Lagoon (Hérault, France), a hot spot of marine species introduction in Europe. Oceanologica Acta **24**:29-49.

Verlaque, M., P. M. Brannock, T. Komatsu, M. Villalard-Bohnsack, & M. Marston (2005): The genus *Grateloupia* C. Agardh (Halymeniaceae, Rhodophyta) in the Thau Lagoon (France, Mediterranean): a case study of marine plurispecific introductions. Phycologia **44**(5):477-496.

Vermeij, G. J. (1991): Anatomy of an Invasion: The Trans-Arctic Interchange. Paleobiology **17**(3):281-307.

Viola, H. J. & C. Margolis (1991): Seeds of Change: Five Hundred Years Since Columbus. –Smithsonian, Washington, DC.

Wares, J. P. & C. W. Cunningham (2001): Phylogeography and Historical Ecology of the North Atlantic Intertidal. Evolution **55**(12):2455-2469.

Watson K., Cheney D. and I. Levine (1998): Can the aquacultured non-indigenous red alga *Porphyra yezoensis* recruit in Eastport, Maine? J. Phycol. **34** (suppl.): 62.

Watson K., Levine I. and Cheney D.P. (1999): Biomonitoring of an aquacultured introduced seaweed, *Porphyra yezoensis* (Rhodophyta, Bangiophycidae) in Cobscook Bay, Maine, USA. -In: Pederson J. (ed.): Marine Bioinvasions, Proceedings of the First National Conference, January 24-27, 1999: 260-264. Massachusetts Institute of Technology Sea Grant College Program, Cambridge, MA.

West A.L., A.C. Mathieson, A. S. Klein, C. D. Neefus & T. L. Bray (2005): Molecular ecological studies of New England species of *Porphyra* (Rhodophyta, Bangiales). Nova Hedwigia **80**:1-24.

Wilkes, R. J., C. Yarish & G. G. Mitman (1999): Observations on the chromosome numbers of *Porphyra* (Bangiales, Rhodophyta) populations from Long Island Sound to the Canadian Maritimes. Algae **14**: 219-222.

William, G. M., D. L. Martin, D. L. Felder, V. L. Asper, & H. M. Perry (2003): Ecological and economic implications of a tropical jellyfish invader in the Gulf of Mexico. Bio. Invasions **5**(1-2):53-69.

Woelkerling, W. J. (1975): On the epibiotic and pelagic Chlorophyceae, Phaeophyceae, and Rhodophyceae of the Western Sargasso Sea. Rhodora **77**:1-40.

Xiao, S., Y. Zang, & R. King (1998): Three-dimensional preservation of algae and animal embryos in a Neoproterzoic phosphorite. Nature **391**:553-558.

Yamazaki, S., Y. Kitade, T. Maruyama & N. Saga (1996): Phylogenetic position of *Porphyra yezoensis* based on the 18S rDNA sequence. J. Marine Biotech. **4**:230-232.

Yarish C., R. J. Wilkes, T. Chopin, X. G. Fei, A. C. Mathieson, A. S. Klein, D. Friel, C. D. Neefus, G. G. Mitman & I. Levine (1998): Domesticating indigenous *Porphyra* (nori) species for commercial cultivation in Northeast America. World Aquaculture **29**:26-29, 55.

Yendo, K. (1902): The distribution of marine algae in Japan. Postelsia **1**:177-192. *pl.* 19-21.

Yoshida T., M. Notoya, N. Kikuehi & M. Mityata (1997): Catalogue of species of *Porphyra* in the world, with special reference to the type locality and bibliography. Nat. Hist. Res., Special Issue No. **3**:5-18.

Zechmann, F. W. & A. C. Mathieson (1985): The distribution of seaweed propagules in estuarine, coastal and offshore waters of New Hampshire, USA. Bot. Mar. **28**:283-294.

APPENDICES

Appendix A. Collection sites, tidal positions, substrata, dates and collectors of specimens examined.

Herbarium #	GenBank #	Identification	Location	Long/Lat	Position	Substrate	Date	Coll:
Porphyra purpurea Type Specimens								
BM000054930A	DQ418732[§], DQ458966[b]	*Porphyra purpurea* neotype	Helgoland, Germany		High Intertidal		17-Oct-1996	A. Wagner
BM000054930B	DQ418733[§], DQ458967[†]	*Porphyra purpurea* isoneotype	Helgoland, Germany		High Intertidal		17-Oct-1996	A. Wagner
Specimens with *rbc* L sequences matching the *Porphyra purpurea* neotype								
WTU330639	DQ458968[†]	*Porphyra rediviva* holotype	Fidalgo Bay, WA, USA	N48.4666, W122.583	High Intertidal	rock	2-Dec-1994	J. W. Stiller
WTU330640	DQ418734[§], DQ458969[†]	*Porphyra rediviva* isotype	Fidalgo Bay, WA, USA	N48.4666, W122.583	High Intertidal	rock	2-Dec-1994	J. W. Stiller
WTU330641	DQ418735[§], DQ458970[†]	*Porphyra rediviva* isotype	Fidalgo Bay, WA, USA	N48.4666, W122.583	High Intertidal	rock	2-Dec-1994	J. W. Stiller
NHA68861	DQ418736[§]	*Porphyra purpurea*	Salthill Beach, Galway, Ireland	N53.266, W9.05	Mid Intertidal	fucoids	9-Sep-1997	R. Wilkes
NHA76737	DQ418737[§], DQ458971[†] DQ535256[‡]	*Porphyra purpurea*	Blomidan Beach, Avonport, Nova Scotia, Canada	N45.256, W64.354	Mid Intertidal	rock	21-Jul-2002	T. Bray
NHA76738	DQ418738[§], DQ458972[†]	*Porphyra purpurea*	Blomidan Beach, Avonport, Nova Scotia, Canada	N45.256, W64.354	Mid Intertidal	rock	21-Jul-2002	T. Bray
NHA77774	DQ418739[§]	*Porphyra purpurea*	Chance Harbor, New Brunswick, Canada	N45.123, W66.352	Mid Intertidal	rock	14-Nov-2003	T. Bray & J. Day
NHA77988	DQ418743[§], DQ458974[†] DQ535254[‡]	*Porphyra purpurea*	Great Island, ME, USA	N43.858 W69.913	Low Intertidal	rock	19-Mar-2005	T. Bray & J. Day

Appendix A. Continued.

Herbarium #	GenBank #	Identification	Location	Long/Lat	Position	Substrate	Date	Coll:
NHA78071	DQ418744[§]	*Porphyra purpurea*	Orr's Island, ME, USA	N43.795, W69.945	Mid Intertidal	rock	17-Apr-2005	T. Bray & J. Day
NHA77846	DQ418745[§]	*Porphyra purpurea*	Back River, Arrowsic, ME, USA	N43.669, W70.241	Sub tidal	*Ascophyllum*	11-Jun-2005	T. Bray
NHA77977	DQ423782[§]	*Porphyra purpurea*	E. End Beach, Portland, ME, USA	N43.669, W70.241	Low Intertidal	rock	18-Mar-2005	T. Bray & J. Day
NHA77978	DQ423783[§], DQ458975[†]	*Porphyra purpurea*	E. End Beach, Portland, ME, USA	N43.669, W70.241	Low Intertidal	rock	18-Mar-2005	T. Bray & J. Day
NHA77980	DQ423784[§]	*Porphyra purpurea*	(Benny Clam Shack) Fore River,	N43.642, W70.284	Subtidal	drift	18-Mar-2005	T. Bray & J. Day
NHA77848	DQ423785[§]	*Porphyra purpurea*	York River, Kittery, ME, USA	N43.141 W70.692	High Intertidal	rock	15-Jun-2004	T. Bray & J. Day
NHA77855	DQ423786[§]	*Porphyra purpurea*	Dover Point, NH, USA	N43.119, W70.827	High Intertidal	rock	10-Jul-2004	T. Bray
NHA78147	DQ418746[§]	*Porphyra purpurea*	Dover Point, NH, USA	N43.119, W70.827	Low Intertidal	rock	10-Feb-2004	C. Neefus
NHA78148	DQ418747[§]	*Porphyra purpurea*	Dover Point, NH, USA	N43.119, W70.827	High Intertidal	rock	13-Feb-2004	C. Neefus
NHA78038	DQ418748[§]	*Porphyra purpurea*	Humarock, MA, USA	N42.137, W70.694	Mid Intertidal	rope	9-Apr-2005	T. Bray & J. Day
Specimens with the alternate *rbc* L sequence (i.e. a single transition from G to T at position 1299)								
GIH/HMSC1319	DQ406592[§], DQ458976[†] DQ535259[‡]	*Porphyra rediviva* [1]	Yaquina Bay, OR, USA	N44.616, W124.033	Drift		6-Oct-2000	K. Dobson

Appendix A. Continued.

Herbarium #	GenBank #	Identification	Location	Long/Lat	Position	Substrate	Date	Coll:
GIH/HMSC1658	DQ406593[§], DQ458977[†]	*Porphyra rediviva* [1]	Yaquina Bay, OR, USA	N44.616, W124.033	Drift		5-Mar-2004	G. I. Hansen
NHA77885	DQ423787[§], DQ458978[†], DQ535260[‡]	*Porphyra purpurea*	Great Island, ME, USA	N43.858, W69.913	Mid Intertidal	rock	15-Jan-2005	J. Day & T. Day
NHA77987	DQ406594[§]	*Porphyra purpurea*	Great Island, ME, USA	N43.858 W69.913	Low Intertidal	rock	19-Mar-2005	T. Bray & J. Day
NHA77999	DQ406595[§]	*Porphyra purpurea*	Great Island, ME, USA	N43.858, W69.913	Low Intertidal	rock	1-Apr-2005	T. Bray & J. Day
NHA77975	DQ406596[§]	*Porphyra purpurea*	E. End Beach, Portland, ME, USA	N43.669 W70.241	Mid Intertidal	*Fucus*	18-Mar-2005	T. Bray & J. Day
NHA77976	DQ406597[§]	*Porphyra purpurea*	E. End Beach, Portland, ME, USA	N43.669, W70.241	Low Intertidal	rock	18-Mar-2005	T. Bray & J. Day
NHA77836	DQ406598[§]	*Porphyra purpurea*	(Benny Clam Shack) Fore River, Portland, USA	N43.642, W70.284	Subtidal	*Dumontia*	27-May-2005	T. Bray
NHA78001	DQ406599[§]	*Porphyra purpurea*	Weymouth Back River, Quincy, MA, USA	N42.246, W70.931	High Intertidal	rock	5-Apr-2005	T. Bray & J. Day
NHA78002	DQ423788[§], DQ458979[†]	*Porphyra purpurea*	Weymouth Back River, Quincy, MA, USA	N42.246, W70.931	High Intertidal	rock	5-Apr-2005	T. Bray & J. Day

Appendix A. Continued.

Herbarium #	GenBank #	Identification	Location	Long/Lat	Position	Substrate	Date	Coll:
NHA78036	DQ406600[§]	*Porphyra purpurea*	Humarock, MA, USA	N42.137, W70.694	Mid Intertidal	rope	9-Apr-2005	T. Bray & J. Day
NHA78039	DQ406601[§], DQ458980[†]	*Porphyra purpurea*	Powder Pt. Duxbury, MA, USA	N42.046 W70.651	Mid Intertidal	rock	9-Apr-2005	T. Bray & J. Day
NHA77898	DQ406602[§] DQ535258[‡]	*Porphyra purpurea*	Hammonasset State Park, CT, USA	N41.249, W72.545	Mid Intertidal	rock	20-Jan-2005	T. Bray & J. Day

[§] *rbc* L sequence

[†] ITS1 sequence

‡ 18s sequence

[1] Pacific specimens that were originally identified as *Porphyra rediviva*

Appendix B. Molecullary confirmed collection information and GenBank accession numbers for *Porphyra yezoensis* and *P. katadae.*

Genotype	Location	Date	Collector	NHA	GenBank Accession # rbcL	ITS1	ID
Porphyra yezoensis f. *yezoensis*							
	Small bridge at East Boothbay, ME	4/6/2005	T. Bray, J. Day	78016	DQ535202	DQ649366	623
	N43.865 W69.585	4/6/2005	T. Bray, J. Day	78017	DQ535203	DQ649367	624
		4/6/2005	T. Bray, J. Day	78025	DQ535204		634
	Samoset Rd, Sawyer Island, Boothbay ME	4/6/2005	T. Bray, J. Day	78026	DQ535205		635
	N43.865 W69.665	4/6/2005	T. Bray, J. Day	78027	DQ535206		636
	New Meadows River, Bath, ME	5/2/2004	T. Bray	77840	DQ535207		390
	N 43.911 W69.868	5/2/2004	T. Bray	77842	DQ535208	DQ64368	392
		5/2/2004	T. Bray	77844	DQ535209		398
	Great Island, Highway 24 bridge, Brunswick, ME	4/1/2005	T. Bray, J. Day	77996	DQ535210		599
	N43.858 W69.913	4/1/2005	T. Bray, J. Day	77998	DQ535211		601
	Orr's Island bridge, Brunswick, ME				DQ535212		694
	N43.795 W69.945						
	Benny's Clam Shack, Fore River, Portland, ME	3/18/2005	T. Bray, J. Day	77981	DQ535213	DQ649370	582
	N43.642 W70.284	3/18/2005	T. Bray, J. Day	77984	DQ535214		585
	Seapoint, Kittery, ME			77864		DQ649392	
	N43.141 W70.692						
	Dover Point, Dover, NH	3/27/1999	A. West	71760	AF271075	AY568277	
	N43.119 W70.827	5/17/2004	T. Bray	77825	DQ535215		373
		5/17/2004	T. Bray	77829	DQ535216		377
		5/17/2004	T. Bray	77832	DQ535217		380
		5/17/2004	T. Bray	77835	DQ535218		383
		1/14/2005	T. Bray	77869	DQ535219		436
		1/14/2005	T. Bray	77870	DQ535220		437
		1/14/2005	T. Bray	77872	DQ535221		439
		2/13/2005	C.D. Neefus	78152		DQ649372	1810
		2/13/2005	C.D. Neefus	78153		DQ649373	1812
		2/13/2005	C.D. Neefus	78154		DQ649374	1813
		2/13/2005	C.D. Neefus	78155		DQ649371	1805
		2/13/2005	C.D. Neefus	78156		DQ649375	1815

Appendix B. Continued.

Genotype	Location	Date	Collector	NHA	GenBank Accession # rbcL	ITS1	ID
	Goose Cove, Gloucester, MA	3/3/2005	T. Bray, A.C.Mathieson	77944	DQ53522	DQ649376	536
	N42.650 W70.672	3/3/2005	T. Bray, A.C.Mathieson	77945	DQ53523		537
		3/3/2005	T. Bray, A.C.Mathieson	77946	DQ53524		538
	Stage Fort Park, Gloucester MA	3/25/2004	T. Bray, J. Day	77793	DQ53525	DQ649377	338
	N42.602 W70.678	3/25/2004	T. Bray, J. Day	77795	DQ53526	DQ649378	340
	Pleasure Bay, South Boston, MA	3/19/2005	C.D. Neefus	78134			CDN1
	N42.333 W70.013						
	Yatch club bridge, Hull, MA	4/15/2005	T. Bray, J. Day	78006	DQ53527		610
	N42.307 W70.890	4/15/2005	T. Bray, J. Day	78009	DQ53528	DQ649379	613
		4/15/2005	T. Bray, J. Day	78011	DQ53529		615
		4/15/2005	T. Bray, J. Day	78013	DQ53530		619
	Breakwater, Plymouth, MA						
	N41.964 W70.666	4/25/2004	T. Bray, J. Day	78130	DQ53531		P425-4
		4/25/2004	T. Bray, J. Day	78131	DQ53532		P425-5
		4/25/2004	T. Bray, J. Day	78132	DQ53533		P425-7
		4/25/2004	T. Bray, J. Day	78133	DQ53534	DQ649380	425-10
	East Sandwich, MA	4/4/2004	T. Bray	77810	DQ53535	DQ649381	357
	N41.775 W70.494						
	Falmouth Heights, MA	4/4/2004	T. Bray	77806	DQ53536	DQ649382	351
	N41.545 W70.588	4/4/2004	T. Bray	77808	DQ53537	DQ649383	355
	Point Independence, MA	1/15/2005	T. Bray	77876	DQ53538	DQ649384	443
	N41.739 W70.653						
	Fort Rodman, New Bedford,	4/21/2005	T. Bray	78098	DQ53539		735
	N41.593 W70.900						
	Parker Memorial Park, Branford Pt, Branford CT	3/5/2005	T. Bray	77961	DQ53540		558
	N41.159 W73.186	3/5/2005	T. Bray	77963	DQ53541		560
		3/5/2005	T. Bray	77964	DQ53542	DQ649385	561

Appendix B. Continued.

Genotype	Location	Date	Collector	NHA	GenBank Accession # rbcL	ITS1	ID
	Lighthouse Point, New	3/5/2005	T. Bray	77947	DQ53543		539
	N41.247 W72.903	3/5/2005	T. Bray	77949	DQ53544	DQ649386	544
		3/5/2005	T. Bray	77952	DQ53545		547
		3/5/2005	T. Bray	77959	DQ53546		555
	Seaside Park, Lighthouse Point, Bridgeport, CT	2/20/2005	T. Bray	77937	DQ535251	DQ649387	525
	N41.142 W73.228						
	Shippan Point, Stamford CT	4/28/2004	C.D. Neefus	77136	DQ53547		CDN 1831
	N41.041 W73.501						
	Playland Park pier, Rye, NY	2/19/2005	T. Bray	77928	DQ53548	DQ649388	515
	N40.966 W73.661	2/19/2005	T. Bray	77930	DQ53549	DQ649389	517
		2/19/2005	T. Bray	77931	DQ53550	DQ649390	518
		2/19/2005	T. Bray	77933	DQ53552		520
		2/19/2005	T. Bray	77934	DQ53553	DQ649391	521
		4/1/1969	M. Wynne				
	Port Isabel, TX			CH2396	DQ813646	DQ813582	PA-1
	N26.073 W87.208						
		1/20/1969	P. Edwards	BM000			
	SW Jetty, Port Aransas, TX			806073			
		3/10/2005	T. Bray				
	N27.834 W97.050			77967	DQ813643	DQ813579	566
	San Jacinto Park,	3/11/2005	T. Bray				
	Galveston, TX			77970	DQ813644	DQ813580	570
	N29.297 W94.793	3/11/2005	T. Bray	77972	DQ813645	DQ813581	573
	Quindao, China			78164	DQ649353		PWYC
	China			78165			95697
Porphyra yezoensis f. *narawaensis*							
	Westport, MA	2/9/2000	C.D. Neefus	78127	DQ630015	DQ649343	WM-3
	N41.514 W71.068						
	Mackerel Cove, Jamestown,	2/8/2005	T. Bray, J. Day	77923	DQ630016	DQ649344	510
	N41.488 W71.380	2/8/2005	T. Bray, J. Day	77925	DQ630017		512
		2/8/2005	T. Bray, J. Day	77926	DQ630018	DQ649345	513
	Charlestown Breachway, Charlestown, RI	4/19/2005	T. Bray	78077	DQ630019	DQ649346	706
	N41.356 W71.639	4/19/2005	T. Bray	78081	DQ630020	DQ649347	711

Appendix B. Continued.

Genotype	Location	Date	Collector	NHA	GenBank Accession # rbcL	ITS1	ID
	Winnapaug Pond, Westerly,	4/19/2005	T. Bray	78085	DQ630021		715
	N41.330 W71.763	4/19/2005	T. Bray	78086			716
		4/19/2005	T. Bray	78089	DQ630022	DQ649348	724
		4/19/2005	T. Bray	78090			725
	Millstone Point, Groton CT			78156	DQ630029	DQ649349	1
				78157	DQ630023	DQ649350	
	N41.304 W72.165						10
	Rocky State Park, Old Lyme, CT	1/20/2005	T. Bray, J. Day	77891	DQ630024		468
	N41.297 W72.246						
	Hammonasset State Park, Madison, CT	1/20/2005	T. Bray, J. Day	77896	DQ630025	DQ649351	474
	N41.249 W72.545	1/20/2005	T. Bray, J. Day	77900	DQ630028	DQ649352	479
		1/20/2005	T. Bray, J. Day	77902	DQ630026		481
Porphyra katadae							
	First Encounter Beach, Eastham, MA	4/1/2000	A.C. Mathueson	72208	DQ630030	DQ649357	
	N 41.960 W 70.02						
	Bone Hill Road, Barnstable, MA	3/27/1999	A.C. Mathueson	69520	DQ630031	DQ649358	
	N41.710 W70.275	3/28/2004	T. Bray	77799			344
		3/28/2004	T. Bray	77800			345
		3/28/2004	T. Bray	77802			347
		3/28/2004	T. Bray	77803			348
		3/28/2004	T. Bray	77804			349
	Bone Hill Road, Barnstable, MA						
	N41.710 W70.275	3/28/2004	T. Bray	77805			350
		1/3/2005	T. Bray	77866	DQ630032		433
		4/9/2005	A.C. Mathieson	78041			651
		4/9/2005	A.C. Mathieson	78042			652
		4/9/2005	A.C. Mathieson	78043			653
		4/9/2005	A.C. Mathieson	78044			654
		4/9/2005	A.C. Mathieson	78045			655
		4/9/2005	A.C. Mathieson	78046			656
		4/9/2005	A.C. Mathieson	78047			657

Appendix B. Continued.

Genotype	Location	Date	Collector	NHA	GenBank Accession # rbcL	ITS1	ID
	NE end of Cape Cod Canal, East Sandwich, MA	4/4/2005	T. Bray	77809			356
	N41.775 W70.494	4/4/2005	T. Bray	77811			357
		4/4/2005	T. Bray	77812			358
		4/4/2005	T. Bray	77813			359
		4/4/2005	T. Bray	77814			360
		4/4/2005	T. Bray	77816	DQ630033	DQ649359	363
		4/4/2005	T. Bray	77817	DQ630034	DQ649360	364
		4/4/2005	T. Bray	77819			366
		4/4/2005	T. Bray	77820	DQ630035	DQ649361	367
	Massachusetts Maritime Academy, Buzzards Bay, MA	1/15/2005	T. Bray	77879			446
	N41.740 W70.621	1/15/2005	T. Bray	77880	DQ630036	DQ649362	447
	Charlestown Beach, RI	4/9/2005	T. Bray	78079	DQ630037		709
	N41.356 W71.639	4/9/2005	T. Bray	78080			710
		4/9/2005	T. Bray	78082			712
		4/9/2005	T. Bray	78084	DQ630038		714

Appendix C. Molecularly confirmed collection information and GenBank accession numbers for *Porphyra olivii, P. tsengii, P. stamfordensis, P. spatulata, and P. collinsii.* * holotype. GenBank sequences: a= *rbc* L, b=ITS1, and c=SSU.

NHA	Coll #	GenBank	Name	Location	Long/Lat	Postion	Substrate	Date	Coll:
77994	597	DQ813616[a]	*Porphyra olivii*	New Meadows River, Brunswick, ME, USA	N43.911 W69.868	Sub	Fucus	1 April, 2005	T. Bray & J. Day
78064	686	DQ813621[a]	*Porphyra olivii*	New Meadows River, Brunswick, ME, USA	N43.911 W69.868	Sub	Gracilaria	17 April, 2005	T. Bray & J. Day
78049	659	DQ813620[a]	*Porphyra olivii*	Pine Pt. Landing, Scarborough, ME, USA	N43.544 W70.332	Mid	rope	12 April, 2005	T. Bray
78138	1806	DQ813625[a] DQ813575[b] DQ813588[c]	*Porphyra olivii*	Dover Point, NH, USA	N43.119 W70.827	Sub	Dumontia	13 Feb., 2004	T. Bray
78149	1816	DQ813626[a]	*Porphyra olivii*	Dover Point, NH, USA	N43.119 W70.828	Sub	Dumontia	14 Feb., 2004	T. Bray
77939	529	DQ813613[a] DQ813572[b]	*Porphyra olivii*	Goose Cove, Glouchester, MA, USA	N42.650 W70.672	Sub	Fucus & Chondrus	3 March, 2005	T. Bray & A. C. Mathieson
77941	532	DQ813614[a] DQ813573[b]	*Porphyra olivii*	Goose Cove, Glouchester, MA, USA	N42.650 W70.672	Sub	Fucus & Chondrus	3 March, 2005	T. Bray & A. C. Mathieson
77943	535	DQ813615[a]	*Porphyra olivii*	Goose Cove, Glouchester, MA, USA	N42.650 W70.672	Sub	Fucus & Chondrus	3 March, 2005	T. Bray & A. C. Mathieson
78007	611	DQ813618[a]	*Porphyra olivii*	Yacht Club, Hull, MA, USA	N42.307 W70.890	Sub	Chondrus	5 April, 2005	T. Bray & J. Day
78037	647	DQ813619[a]	*Porphyra olivii*	Humarock, MA, USA	N42.137 W70.694	Sub	Chondrus	9 April, 2005	T. Bray & J. Day
77864	431	DQ813610[a]	*Porphyra olivii*	Bone Hill Rd. E. Barnstable, MA, USA	N41.710 W70.275	Mid	Gracilaria	3 Jan., 2005	T. Bray

Appendix C. Continued.

NHA	Coll #	GenBank	Name	Location	Long/Lat	Postion	Substrate	Date	Coll:
78096	733	DQ813624[a]	*Porphyra olivii*	(boat ramp) New Bedford, MA, USA	N41.614 W70.909	Sub	Cement	21 April, 2005	T. Bray
78073	702	DQ813622[a]	*Porphyra olivii*	Judith Pt., Sand Hill State Park, RI, USA	N41.376 W71.513	Sub	Chondrus	19 April, 2005	T. Bray
78075	704	DQ813623[a]	*Porphyra olivii*	Judith Pt., Sand Hill State Park, RI, USA	N41.376 W71.513	Sub	Chondrus	19 April, 2005	T. Bray
77899	478	DQ813611[a] DQ813570[b] DQ813587[c]	*Porphyra olivii*	Hammonasset State Park, CT, USA	N41.249 W72.54	Mid	Fucus	20 Jan., 2005	T. Bray & J. Day
77936	524	DQ813612[a] DQ813571[b]	*Porphyra olivii*	Seaside Park, Bridgeport, CT, USA	N41.14279 W73.22831	Mid	rock	20 Feb., 2005	T. Bray
78115	1866	DQ813631[a]	*Porphyra olivii*	Cove Is, Stamford, CT, USA	N41.041 W73.501	Sub	Chondrus	30 Jan., 2003	C. Neefus
78116	1821	DQ813627[a]	*Porphyra olivii*	Cove Is, Stamford, CT, USA	N41.041 W73.501	Sub	Chondrus	16 Feb., 2004	C. Yarish
78117	1823	DQ813629[a]	*Porphyra olivii*	Cove Is, Stamford, CT, USA	N41.041 W73.501	Sub	Chondrus	16 Feb., 2004	C. Yarish
78118	1822	DQ813628[a]	*Porphyra olivii*	Cove Is, Stamford, CT, USA	N41.041 W73.501	Sub	Chondrus	16 Feb., 2004	C. Yarish
78119	1824	DQ813630[a]	*Porphyra olivii*	Shipham Pt. Stamford, CT, USA	N41.021 W73.521	Low	Chondrus	16 Feb., 2004	C. Yarish
77785	330	DQ813585[c] DQ813607[a]	*Porphyra tsengii*	New Meadows River, Brunswick, Maine, USA	N43.911 W69.868	Sub	Gracilaria	12 March, 2004	T. Bray & A. C. Mathieson
77786*	331	DQ813586c DQ813567[b]	*Porphyra tsengii*	New Meadows River, Brunswick, Maine, USA	N43.911 W69.868	Sub	Fucus	12 March, 2004	T. Bray & A. C. Mathieson
77792	337	DQ813608[a] DQ813568[b]	*Porphyra tsengii*	New Meadows River, Brunswick, Maine, USA	N43.911 W69.869	Sub	Fucus	12 March, 2004	T. Bray & A. C. Mathieson
78066	688	DQ813609[a]	*Porphyra tsengii*	New Meadows River, Brunswick, Maine, USA	N43.911 W69.868	Mid	Fucus	17 April, 2005	T. Bray & J. Day

Appendix C. Continued.

NHA	Coll #	GenBank	Name	Location	Long/Lat	Postion	Substrate	Date	Coll:
		DQ813636[a]							
		DQ813577[b]			N41.545				
77883	456	DQ813591[c]	*Porphyra stamfordensis*	Falmouth, MA, USA	W70.588	Mid	rock	15 Jan., 2005	T. Bray
				(boat ramp) Westport, MA,	N41.514				T. Bray & J.
77921	508	DQ813640[a]	*Porphyra stamfordensis*	USA	W71.068	Mid	Fucus	8 Feb., 2005	Day
				Attawan Beach, Niantic, CT,	N41.302				T. Bray & J.
77888	461	DQ813637[a]	*Porphyra stamfordensis*	USA	W72.204	Mid	rock	20 Jan., 2005	Day
				Hammonasset State Park,	N41.249				T. Bray & J.
77895	473	DQ813638[a]	*Porphyra stamfordensis*	CT, USA	W72.545	High	rock	20 Jan., 2005	Day
		DQ813639[a]							
		DQ813578[b]		Hammonasset State Park,	N41.249				T. Bray & J.
77901	480	DQ813592[c]	*Porphyra stamfordensis*	CT, USA	W72.545	Mid	Fucus	20 Jan., 2005	Day
					N41.021		rock &		C. Neefus &
78113*	1863	DQ813641[a]	*Porphyra stamfordensis*	Stamford, CT, USA	W73.521	High	Fucus	18 Dec., 2004	A. Neefus
					N41.021		rock &		
78140	CTCI1	DQ813642[a]	*Porphyra stamfordensis*	Stamford, CT, USA	W73.521	Mid	Fucus		C. Yarish
				Powder Pt. Duxbury, MA,	N42.046				T. Bray & J.
78031	640	DQ813635[a]	*Porphyra spatulata*	USA	W70.651	Drift		9 April, 2005	Day
		DQ813633[a]		Powder Pt. Duxbury, MA,	N42.046				T. Bray & J.
78032	641	DQ813590[c]	*Porphyra spatulata*	USA	W70.651	Drift		9 April, 2005	Day
		DQ813632[a]		(boat ramp) Westport, MA,	N41.514				T. Bray & J.
77916*	501	DQ813589[c]	*Porphyra spatulata*	USA	W71.068	Sub	Chondrus	8 Feb., 2005	Day
				(boat ramp) Westport, MA,	N41.514				T. Bray & J.
77917	502	DQ813634[a]	*Porphyra spatulata*	USA	W71.068	Sub	Chondrus	8 Feb., 2005	Day
				(boat ramp) Westport, MA,	N41.514				T. Bray & J.
77919	504	DQ813576[b]	*Porphyra spatulata*	USA	W71.068	Sub	Chondrus	8 Feb., 2005	Day
		DQ813617[a]			N42.307				T. Bray & J.
78005	609	DQ813574[b]	*Porphyra collinsii*	Yacht Club, Hull, MA, USA	W70.890	Sub	Chondrus	5 April, 2005	Day
				Bone Hill Rd. E. Barnstable,	N41.710				
77861	428	DQ813595[a]	*Porphyra collinsii*	MA, USA	W70.275	Mid	Gracilaria	3 Jan., 2005	T. Bray

Appendix C. Continued.

NHA	Coll #	GenBank	Name	Location	Long/Lat	Postion	Substrate	Date	Coll:
77862	429	DQ813596[a] DQ813597[a]	*Porphyra collinsii*	Bone Hill Rd. E. Barnstable, MA, USA	N41.710 W70.275	Mid	Gracilaria	3 Jan., 2005	T. Bray
77864	431	DQ813569[b] DQ813598[a] DQ813566[b]	*Porphyra collinsii*	Bone Hill Rd. E. Barnstable, MA, USA	N41.710 W70.275	Mid	Gracilaria	3 Jan., 2005	T. Bray
77878	445	DQ813584[c]	*Porphyra collinsii*	Maritime Academy, Buzzard Bay, MA, USA	N41.740 W70.621	Sub	Dumontia	15 Jan., 2005	T. Bray
77955	551	DQ813599[a]	*Porphyra collinsii*	Lighthouse Pt., New Haven, CT, USA	N41.247 W72.903	Mid	Fucus & Chondrus	5 March, 2005	T. Bray
77957	553	DQ813600[a]	*Porphyra collinsii*	Lighthouse Pt., New Haven, CT, USA	N41.247 W72.903	Drift		5 March, 2005	T. Bray
77958	554	DQ813601[a]	*Porphyra collinsii*	Lighthouse Pt., New Haven, CT, USA	N41.247 W72.904	Drift		5 March, 2005	T. Bray
78100	738	DQ813602[a] DQ813594[a] DQ813565[b]	*Porphyra collinsii*	(boat ramp) Westport, MA, USA	N41.514 W71.068	Sub	Chondrus	21 April, 2005	T. Bray
78139*	20	DQ813583[c]	*Porphyra collinsii*	Millstone Pt., CT, USA	N41.304 W72.165	Low		23 April, 2004	A.C. Mathieson
78157	6	DQ813593[a]	*Porphyra collinsii*	Millstone Pt., CT, USA	N41.304 W72.165	Low		23 April, 2004	A.C. Mathieson
78163	CT5b	DQ813605[a]	*Porphyra collinsii*	Cove Is, Stamford, CT, USA	N41.041 W73.501			18 Sept., 1997	A.C. Mathieson
78164	MA131	DQ813606[a]	*Porphyra collinsii*	Westport, CT, USA	N41.514 W71.068			27 April, 1997	C. Yarish
8560	8560	DQ813603[a]	*Porphyra collinsii*	(off shore) Milford, CT, USA				8 May, 1967	E. Hehre
BM0008 06074	BM074	DQ813604[a]	*Porphyra collinsii*	Chesapeake Bay, VA, USA			Gracilaria	19 March, 1971	Ott